Biological studies of the river lamprey (Lampetra fluviatilis L.) related to the neurophysiology of olfaction.

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BIOLOGICAL STUDIES OF THE RIVER LAMPREY *(Lampetra fluviatilis* L.) RELATED TO THE NEUROPHYSIOLOGY OF OLFACTION

Submitted by

R. J. HUGGINS

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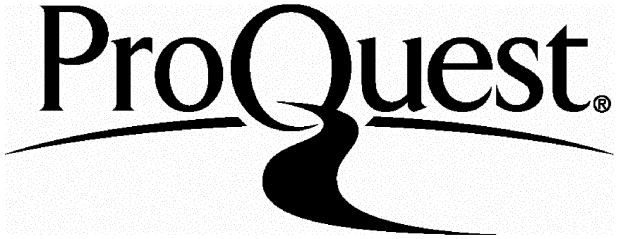
R. J. Huggins



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SUMMARY

The biology of the River lamprey (*L. fluviatilis* L.) was investigated by relating ecological observations to neurophysiological studies of olfaction.

The larval life of *L. fluviatilis* in British rivers is estimated as 4½ years, and the morphology of spring-caught downstream migrants is compared with that of newly transformed animals. A high proportion of downstream migrants were successfully acclimated to high salinities but could not be induced to feed under laboratory conditions.

It is postulated that high autumnal river flows direct the movements of upstream migrants and the hydrographic conditions for spawning are described. The possibility of hybridisation with *L. planeri*, and the accumulation of lampreys on the spawning grounds are discussed.

The brain activity of lampreys was related to that of other vertebrates and the olfactory brain response to stimulation with dissolved chemicals, "home" and other natural waters was explored. The stimulant effect of river water was enhanced if it had contained lampreys, and the electrophysiological results are discussed in relation to homing and other aspects of the biology of the River lamprey.

I INTRODUCTION

II THE BIOLOGICAL STUDIES

A) INTRODUCTION:

i) The Larval Phase

ii) Metamorphosis and Downstream Migration

iii) The Adult Feeding Phase

iv) The Upstream Migration

v) The Spawning Phase

B) EXPERIMENTAL WORK:

i) The Length of Larval Life

ii) The Capture, Salinity Tolerance and Feeding Behaviour of Downstream Migrants

iii) Factors Affecting the Capture of Upstream Migrants

iv) Spawning Conditions and Sites

v) Morphometric Studies of Spent *L. fluviatilis*

C) DISCUSSION

III THE ELECTROPHYSIOLOGICAL STUDIES

A) INTRODUCTION:

i) Brain Activity in Vertebrates

ii) Olfactory Lobe Activity and Homing in Salmon

iii) Theories of Olfaction

iv) The Structure of the Olfactory System

v) The Electrophysiology of the Olfactory System

vi) Olfactory Discrimination

vii) The Olfactory System of the Lamprey

viii) Olfaction and Behaviour in Lampreys

ix) Olfactory Thresholds

B) MATERIALS AND METHODS:

i) Recording Technique

ii) The Electrode

iii) The Recording Apparatus

iv) The Stimulator

v) Maintenance of the Preparation

vi) The Dissection and Experimental Procedure

vii) Preparation of Stimulant Solutions

III continued

C) RESULTS

i) Brain Activity in Lampreys

ii) Optic Tectum Responses to Photic Stimulation

iii) Olfactory Lobe Activity and Temperature

iv) Olfactory Lobe Activity and Respiration

v) Olfactory Lobe Responses of Upstream Migrant *L. fluviatilis*

Responses to Stimulation with Solutions of 1,2-diaminoethane

Responses to Stimulation with Solutions of Isoleucine Methyl Ester

Responses to Stimulation with Solutions of Sodium Chloride

vi) Changes in Olfactory Lobe Activity After Epithelial Stimulation

vii) Olfactory Lobe Responses to Stimulation by Home and Other Natural Waters

D) DISCUSSION

IV CONCLUSIONS

REFERENCES

* * * * * * *

Some of the work described in this thesis has already been published, and copies of the papers, whose titles are shown below, are included at the end of the thesis.

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I

INTRODUCTION

I INTRODUCTION

The lampreys *(Petromyzonidae)* and the salmonid fish *(Salmonidae)* exhibit ecological similarities (Tate Regan, 1911; Berg, 1935; Fontaine, 1948), despite the fact that they are representative of widely separated taxonomic groups. Berg (1935) compared the life cycles of the River lamprey *(Lampetra fluviatilis)* and the Sea trout *(Salmo trutta)*. He recorded similarities in the phases of their life cycles, and in their distributions. He further noted that both species are each separately closely related to a dwarf non-migratory form, and both River lamprey and Sea trout populations include some small migratory members which spawn after a reduced feeding phase. Landlocked populations of both species were also recorded. These ecological parallels have been variously interpreted as a consequence of a host/parasite relationship (Abakumov, 1960) or as a result of the operation of common environmental factors (Gritsenko, 1968).

One aspect of migratory salmonid ecology, namely homing, has attracted scanty attention from researchers working on lampreys. Homing of salmonids, or the return of adults to their natal* tributary in order to spawn after a long and distant sojourn in the sea, is thought to be of selective advantage to fish populations, (Wynne-Edwards, 1962), and requires definitive sensory mechanisms (Hasler, 1966). It is of interest therefore, to compare the importance of homing, if any, in the ecology of both salmonids and lampreys.

*In accordance with previous work on homing, the term natal is used in this text to signify the stream in which a salmonid fish hatched and spent the early years of its life.

That salmon return to their natal stream to spawn has been postulated for many years, and Walton (1653), recording possibly the first fish-tagging experiments, stated that salmon with ribbons or threads tied in their tails had returned to the same rivers in which they had been bred. The homing legend has been proved to be true by recent work both in the laboratory and the field, (for reviews see Harden-Jones, 1968 ; Hasler, 1966). After long and wide-ranging marine movements, salmon return via a sun-compass method of orientation (Fraenkel and Gunn, 1940; Hasler, 1966), to the mouth of their natal river. They enter the river, and select at each division of the stream, tributaries which will finally lead them to their natal tributary, where they spawn. Tributary selection has been shown to be dependant on the olfactory sense of the salmon. The salmon is believed to be 'imprinted' in its pre-smolt stages with the smell or 'bouquet' of its natal tributary, and it is this mechanism which provides the basis for its later ability to recognise and return to the same stream. In addition, because natal tributary water is diluted in the river system, it is postualted that a series of odours are sequentially imprinted as the salmon smolt migrates downstream. Thus a series of attractive smells provides a trail which is followed through the river system on the return upstream migration.

The sensory mechanisms necessary for tributary homing must be well developed and very sensitive. What selective factors could be involved in the development of these mechanisms? The spawning preference of salmon for river tributaries may at least be partly related to the fact that this area provides a satisfactory environment for the development of embryonic and young salmon. Tributary water is usually

well oxygenated, and in comparison with the marine environment, the spawning areas in rivers may have a reduced number of predators and parasites. Because of tributary homing, geographical isolation of population is achieved resulting in a consistent population/environment relationship which leads to an equilibrium between the size of the population and the resources of the habitat (Twitty 1959). In migratory fish, an ecological homeostasis of this type restricts the offspring population to a level where the resources of the nursery area can be optimally utilized and leaves the migratory adult stock free to exploit other habitats (Nikol'skii, 1963).

Inbreeding and restricted population size caused by geographical isolation is thought (Hasler 1966) to favour selective adaptation leading to greater survival within the traditional habitat and fixation of morphometric and meristic characters leading to eventual speciation. However, homing could be harmful to a population if conditions permitted spawning but did not permit successful development of offspring. Total homing of a population to an increasingly hostile traditional habitat could lead to extinction, and factors such as pollution, dams, landslides or extreme flooding could all have disastrous effects on a homing population. Wynne-Edwards (1962) suggested that the only biological insurance open to a homing species must be pioneering members within populations which are capable of adapting to new environments, while a multiplicity of spawning grounds favours the survival of a homing species.

Gerking (1959) defined homing as "the return to a place formerly occupied instead of going to other equally probable places", and by this definition, many animals can be said to home, including

limpets (Pieron, 1909), bees (von Frisch, 1927), frogs (Breder, 1925), newts (Twitty, 1959), turtles (Carr, 1965), birds (Matthews, 1953) and mammals (Schmid, 1932). Attempts to investigate possible homing behaviour in lampreys have involved 'mark and recapture' experiments on upstream migrating lampreys. No experiments based on returns of tagged downstream migrants have been recorded.

Applegate and Smith (1951) found that although several rivers running into the Great Lakes system were blocked near their mouths by hydroelectric power dams, they still attracted large numbers of landlocked Sea lampreys *(Petromyzon marinus)* during their spawning migration. Thus although there were no spawning sites available in the short stretch of river which was accessible to lampreys, the lamprey run into the Cheboygan River from Lake Huron was conservatively estimated at 40,000 migrants. Unfortunately Applegate and Smith (1951) do not give the date of construction of the dam, nor whether it could be passed by downstream migrant lampreys. The large spawning run does however indicate that the Cheboygan River was in some way attractive to many sexually maturing lampreys, and the authors state that "field experiments, anatomical studies and other observations seemed to indicate that many of these blocked individuals do not locate suitable streams in which to spawn, and ultimately die in the lake proper without spawning".

Nearly 3,000 lampreys were trapped at the base of the dam and tagged and released. Of these tagged animals, only 289 (10%) were recaptured, and of these, 86.9% were caught in nearby rivers (30 miles) while 10.6% were caught nearby in the Lake (within 23 miles) during a period of 65 days from the date of release. From these results it was

inferred that 90% of the estimated 40,000 migrants in the Cheboygan River spawning-run either died without spawning or less probably spawned in other rivers far from the central zone. The important factors which affected the numbers of diverted lampreys entering other streams were the distance of the alternative streams from the point of release (56.9% in streams within 12 miles), and the size of the stream (Ocqueoc River, 30 miles from the release point is a large unobstructed stream which attracted 24.5% of the diverted migrants).

Following the work of Applegate and Smith (1951), Skidmore (1959) attempted some further experiments on the migrations of landlocked Sea lampreys in the Great Lakes. A small sample (159) of migrants was caught from the Pancake River and tagged. They were then released in groups at a series of locations within 5 miles of the shore in the area of the mouths of the Pancake, Carp and Batchawana Rivers. 33 of the tagged lampreys were recaptured in the rivers, 14 in the Pancake River, 5 in the Carp River, and 14 in the Batchawana River. Tagged lampreys were mainly caught in the river nearest to their point of release. Skidmore observed that 34 recaptures was too small a return to yield a conclusive result.

The work of Abakumov (1956) on the biology of the River lamprey *(L. fluviatilis)* in the Gulf of Riga (Baltic Sea) included a tagging experiment. Abakumov caught and tagged 500 River lampreys from the River Gauja and 300 from the River Daugava. Both rivers flow into the southern Gulf of Riga and their mouths are approximately 15 km. apart. The 500 tagged lampreys from the Gauja were released in groups at distances of 0, 5 km., 10 km., 15 km., and 20 km. from its mouth. All of the 296 recaptured lampreys from these groups were

recaught in the Gauja. The 300 River lampreys from the Daugava were all released at a point equidistant from the mouths of the Daugava and Gauja. The lampreys from this group which were later recaptured were all recaught in the Gauja.

Thus of the 800 tagged and released lampreys, 407 were recaptured in the Gauja and none were recaptured in the Daugava. It appears that although the Daugava is the larger river, the Gauja is a more attractive river for migrating River lampreys, and this is borne out by the fact that the Gauja normally has a large run of River lampreys [1,256,000 in 1903/4 from Abakumov (1956)].

The work of Applegate and Smith (1951), Abakumov (1956) and Skidmore (1959) does not provide evidence for or against homing behaviour in lampreys. However it appears that under certain unknown conditions, some rivers are especially attractive to migrating lampreys.

Work on homing in salmon has continued with extensive field experiments, and more recently, with detailed electrophysiological studies of olfaction related to homing (for review see Hara, 1970). Results have shown that salmon collected from their spawning grounds exhibit stronger electroencephalographic responses in their olfactory bulbs to olfactory stimulation by their home river water, than to stimulation by water from other rivers which are equally suitable for spawning. The waters traversed during the migration also evoke comparatively stronger responses.

As resources were not available to carry out a meaningful tagging experiment on migrating lampreys, it was proposed that electro-encephalographic studies of lampreys, similar to those carried out on

homing salmon, might give some indication of the level of olfactory discrimination in lampreys, and whether they were capable of recognising the water from their spawning sites.

Before undertaking such studies it was necessary to investigate the general biology and ecology of some British River lamprey populations, since little information was available. Thus the work in this thesis is composed of two parts; i) work on the ecology and general biology of River lampreys and ii) electroencephalographic studies of olfaction in migrating River lampreys directed towards an assessment of their level of olfactory discrimination in relation to possible homing.

II

THE BIOLOGICAL STUDIES

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A. INTRODUCTION

Three lamprey species occur in British rivers, the Sea lamprey *(Petromyzon marinus)*, the River lamprey *(Lampetra fluviatilis)* and a brook lamprey *(Lampetra planeri)*. The three species are found in unpolluted running water, and the anadromous Sea and River lampreys can only inhabit rivers in which their upstream migrations are not blocked. The climatic, topographic and hydrographic characteristics of different rivers may modify the basic life history of a lamprey species and produce variations in the timing of the different life-cycle phases, the lengths of migratory routes and the feeding conditions. It is appropriate, therefore, to review the available literature on the life history of the River lamprey within the following framework:

i) the freshwater larval phase;

ii) metamorphosis and downstream migration towards the estuary;

iii) the parasitic feeding phase in brackish or salt water;

iv) upsteam migration towards spawning sites;

and

v) the spawning phase.

i) The larval phase:

After fertilization, the embroyonic and pro-ammocoete stages of development take place in the nest gravel (Whiting, 1948). Young ammocoetes then leave the redd and are carried downstream by the current, to be deposited in areas of 'fine silt', (Applegate, 1950; Abakumov, 1956). Further distribution of ammocoetes in a river system depends on its hydrology and on the activity of the ammocoetes. In rivers and streams of low gradient little further distribution takes place, while in streams characterised by higher flow rates and floods, the distribution of ammocoetes is more extensive (Baxter, 1954). Passive, downstream dispersion of ammocoetes is of major importance, but there is evidence that ammocoetes may, under certain conditions, emerge from their substrate and undertake periods of active swimming during the night (Gritsenko, 1968; Long, 1968). Ammocoetes may actively seek areas of rotting vegetation on the river bed (Enequist, 1937). Stable ammocoete habitats have been described on the basis of current velocity and water depth (Baxter, 1957). Ammocoetes are abundant at the edges of rivers and streams, especially in shaded eddies or areas where there is a reduction in water current velocity. These areas are also often rich in diatoms and desmids which form the major part of ammocoete food (Creaser and Hann, 1929; Schroll, 1957; 1959; Sterba, 1962).

The larval phase is terminated by metamorphosis into an adult form. Estimates of the duration of the larval phase in the River lamprey vary between 3 and 6 years. The technique for aging employed on other fish, such as examination of annular growth rings in bony structures, cannot or have not been employed on lampreys, and all

estimations of the age of ammocoetes have been based on length-frequency distributions. However, even with this method difficulties arise because at present, ammocoetes of the River lamprey cannot be externally distinguished from those of *L. planeri*. It is therefore necessary before ageing ammocoetes of the two species, to resort to the rather tedious process of estimating the number of oocytes which show different mean values in the two species. By this method, relatively pure populations of one species can be identified, which can then be aged by length-frequency analysis.

Meek (1917) used small samples of ammocoetes presumed to be *L. fluviatilis* from the River Tyne, and estimated 3 years as the larval period of this species, a value similar to that obtained by Hubbs (1925) after re-analysis of Meek's results. Privnol'nyev (1964) assessed the larval phase of *fluviatilis* ammocoetes from Latvian rivers at 3 years, although he did not explain his method of separating *fluviatilis* ammocoetes from those of *planeri*. Berg (1935) claimed that the length of larval life in *fluviatilis* was 4 - 5 years, while Abakumov (1957) and Eglite (1958b),working on lampreys from rivers in the Baltic area gave a duration of 5 - 6 years. MacDonald (1959), using ammocoetes collected from Scottish rivers, also estimated the larval phase of *fluviatilis* as 5 years although this figure was based on the technique of initial, and possibly incorrect, allocation of ammocoetes into year classes.

As a period of greatly reduced or arrested growth in the final stages of the larval phase has been postulated by Gage (1928), Leach (1940), Churchill (1947) and Potter (1970), it is important that this factor should be considered when estimating the duration of larval life based on length frequency distributions. An arrested growth phase would, of necessity, extend many previous estimates of larval life by an additional year.

ii) Metamorphosis and downstream migration:

The external metamorphosis involves the development of eyes, a great increase in fin height, the development of a functional suctorial mouth with teeth and changes in skin pigmentation. Internal changes take place over several months, although the external changes are completed with 4 to 5 weeks (Hardisty and Potter, 1971). In common with other Northern Hemisphere lamprey species, metamorphosis of *L. fluviatilis* occurs in the autumn, although the exact timing may depend on local seasonal river temperatures, (Hardisty and Potter, 1971). Completion of metamorphosis is accompanied by a behavioural change from the sedentary burrowing habit of the ammocoete to that of the free-swimming parasitic adult. Since small adults have been captured in estuaries in the spring (Benecke, 1880; Weissenberg, 1925, 1927; Enequist, 1937; Berg, 1948; Bahr, 1952; Zanandrea, 1957), the downstream migration has been thought to occur between late winter and early summer.

iii) The adult feeding phase:

Information is lacking on the marine feeding phase of the River lamprey. The size and gonadal status (Hardisty, 1971) of marine and freshwater specimens caught by Zanandrea (1959) in the Gulf of Gaeta suggest that the population from this area, the duration of the adult phase from metamorphosis to spawning is approximately 2 - 2½ years. However, the size of upstream migrants is related to both the duration of the feeding phase and local feeding conditions, and within European stocks of River lampreys there is great variability in size (Lanzing, 1959; Zanandrea, 1961; Hardisty, 1963).

The dispersion of River lampreys during their marine feeding phase has received little attention, although Zanandrea (1959) considered that the River lamprey of the Gulf of Gaeta was an inshore feeder. Few specimens were found further than 15 km. from the coast, and most were found at depths of 15 - 50 m. Bahr (1952) carried out salinity tolerance tests on small numbers of River lampreys from the Elbe estuary, and concluded that this population fed mainly in brackish water. Small (15 - 20 cm) lampreys survived for more than 6 weeks in 33‰ sea water, but Bahr judged that salinities over 22‰ affected their overall condition. Small numbers of immature River lampreys were caught in the Elbe estuary in all months of the year when the fisheries were not affected by weather conditions (April - November). Catches of lampreys were highest, however, during the period September - November, when the estuarine population was increased due to the presence of large numbers of anadromous migrants.

Records of River lampreys caught at sea are few, probably

because the animal is not trapped by large mesh fishing nets. Schnakenbeck (1929) records one specimen caught near Heligoland at a depth of 40 m. Thus the fragmentary information relevant to the distribution of River lampreys during their marine phase is inconclusive.

Bottom fauna, detritus and fish eggs were commonly claimed to be the major constituents of the diet of adult *L. fluviatilis* (Günther, 1853; Jenkins, 1925; Ehrenbaum, 1936; Bahr, 1952), but more recent observers have emphasised the role of parasitic and predatory feeding on fish. Host fish are said to include the Cod, Herring and Sprat (Bahr, 1933; Eglite, 1958a, b). Tambs-Lyche (1963) recorded the Mackerel as a prey of Scandinavian River lampreys, and the discovery of the nematode *Cystideola carionis* in the gut of feeding River lampreys is in accord with Berg's (1948) statement that River lampreys feed on the Smelt, a species commonly infested with the nematode. Abakumov (1960) recorded that the Salmon was attacked by River lampreys, while Cotronei (1927) found small fish in the alimentary canal of River lampreys. The above reports indicate that *L. fluviatilis*, during its marine phase, feeds on many teleost species.

The feeding of landlocked River lampreys has not been studied in detail, but the Powan *(Coregonus clupeoides)* of Loch Lomond is commonly attacked by River lampreys (Robertson, 1875; Tate Regan, 1911), and in Finnish Lakes the Vendace *(Coregonus albi)* is subject to predation by *L. fluviatilis* (Konni, pers. comm.).

iv) The upstream migration:

The initiation of migration and sexual maturation in *L. fluviatilis* has not been studied, but may, as apparently in *P. marinus*, depend on climatic factors such as photoperiodicity and water temperature (Applegate, 1950; Skidmore, 1959). River lampreys are relatively easily caught during their upstream migration, which provides the reason for the extensive studies on some ecological and physiological aspects of this phase of the life cycle. Most *L. fluviatilis* spawning migrations extend from autumn to spring in Europe, although in Italy few animals are seen before December, and most of the movement into the rivers occurs in February and March (Zanandrea, 1957, 1959). In the River Meuse, the movement of lampreys over a weir, 65 km. from the river mouth, extends from August to January (Lanzing, 1959). Migration into the River Elbe begins in September and extends to December, the peak occuring in October (Tesch, 1967). Migration from the Gulf of Finland into the River Neva commences in August and continues until late May (Ivanova Berg, 1936; Berg, 1948). In this river there are two peaks of migratory activity, one in November and one in early May, and although migratory activity continues throughout the winter, Berg (1935) considers the November and May migrants as distinct races which show anatomical and physiological differences including teeth condition and gonadal development. Rivers of Latvia and Lithuania do not appear to have two clearly defined migratory races (Benecke, 1881; Abakumov, 1961; Gaygalus and Matskevichyus, 1968), although the migrations in these rivers extend from July to the following spring, with a peak of migratory activity in August (Abakumov, 1956, 1961). In the Severn, most migratory activity

occurs in autumn or late winter but in some years migration continues on a small scale into the spring (Hardisty, pers. comm.).

Migrating River lampreys are most active at night (Enequist, 1937; Wikgren,1953; Ryapolova, 1964; Tesch, 1967), and most are caught on dark nights during high water conditions (Seligo, 1926; Bucholtz, 1938). In some commercial lamprey fisheries, animals are caught by partially illuminating the river at night, so that migrants are compelled to enter a dark corridor leading to traps (Abakumov, 1956). Tesch (1967) correlated the size of nightly lamprey catches from the River Elbe with the lunar cycle and indicated that migration through the estuary may be influenced by tidal conditions. Abakumov (1954) reported that hydrometerological factors such as current velocity, water height and turbidity, relative sea and river temperatures, wind, light intensity and the lunar cycle could affect migration into rivers from the Gulf of Riga.

During their spawning migration, lampreys undergo major morphological and physiological changes, including degeneration of the gut (Lanzing, 1959) and depletion of lipid reserves (Bentley and Follett, 1965). As the animals do not feed during their prolonged migration, they utilize their considerable lipid reserves for energy requirements and gonadal development. This leads to an allometric reduction in length (Larsen, 1962), shrinkage being possible because of the absence of bony structures. Comparison of early migrant and spawning River lampreys showed a 23% and 54% reduction in length and weight respectively in females, and comparable values of 27% and 40% in males (Ivanova-Berg, 1933) More rapid shrinkage occurs under hypophysial control during terminal sexual maturation (Larsen, 1969).

Long term fluctuations occur in the numbers of migrating River lampreys, and Abakumov (1956) recorded a 7 - 9 year cycle of abundance in the numbers caught in the River Gauja during the period 1896 - 1911. Very large catches of lampreys in this river in 1944/45 and 1952/53, and relatively small number in 1947 provide further examples of these fluctuations.

v) The Spawning Phase:

The time of River lamprey spawning varies among rivers. In German rivers spawning may occur in late February (Lauterborn, 1926) while in rivers of the Gulf of Finland, spawning has been recorded in June (Berg, 1948; Abakumov, 1956). The temperatures at which River lampreys spawn appear to vary considerably among different rivers. Thus, Lauterborn (1926) records spawning in February in the Upper reaches of the Rhine at 8.7°C, while Hagelin and Stefner (1958) in Norway recorded spawning at 11.0°C in late May. Eglite (1958) recorded a spawning temperature of 9.5°C in Latvian rivers, while Gaygalas and Matskevichyus (1968) found spawning occurring at 15.4 - 17.2°C in Lithuanian rivers. Although water temperature has been shown to be critical in the spawning of *L. planeri* (Hardisty, 1961), the wide range of spawning temperatures recorded for *L. fluviatilis* (8.7 - 17.2°C) suggest either that the relationship between temperature and spawning is variable within the species, or that confusion over the term 'spawning' has led to variable records. The excellent work of Hagelin and Stefner (1958) and Hagelin (1959) fully describes the spawning behaviour of the River lamprey, when observed under laboratory conditions, and should be used as a basis when recording field observations.

Abakumov (1956) recorded spawning of River lampreys at night, whereas all other observations have been made during daylight, and Sterba (1953, 1962) has shown that spawning *L. planeri* distinctly preferred sunlight areas. Lauterborn (1926) made field observations and measurements of River lampreys spawning in the Upper Rhine between February and April. Redds were in flowing water, 30 - 60 cm. deep,

and consisted of rounded depressions in the river bed, 30 - 40 cm. in diameter and 5 - 8 cm. in depth. Spawning *planeri* were observed in redds made and occupied by spawning *fluviatilis*, and Lauterborn discussed the possibility of hybridization under these conditions.

Some phases in the life history of *L. fluviatilis* have thus received attention, although only rarely have they been covered in depth, and results of different workers often show marked variation. Estimates of the length of larval life thus vary, and the marine phase is little documented. The timing of the upstream migration differs, and in some rivers two distinct migratory races have been proposed. Finally the time of and temperature at spawning are not the same among rivers. In this thesis, therefore, the life history of *L. fluviatilis* in the rivers of the Bristol Channel area is described with special emphasis on the length of larval life, the times of downstream and upstream migrations and an assessment of some factors involved in spawning.

B. EXPERIMENTAL WORK

i) The length of larval life:

Several rivers in England and Wales were investigated, and productive ammocoete sites were found on the Rivers Taw (Devon), Teme (Worcestershire) and Tywi (Carmarthenshire). Areas selected for sampling were usually close to road bridges for ease of access, and at most sites large, permanent 'mud' banks were present, such as at Bransford Bridge on the River Teme (Plate 1). All ammocoetes were collected by using a portable electric fish shocker, (Cybertronic Mk.10, Mini Trawl Electrofisher, Marine Electrics), which produced a pulsed direct current at 350 - 400 volts from a 12 volt car battery. Electro fishing was usually carried out when rivers were clear and low, although sampling was still possible at some sites during slight flooding. A cathode of aluminium plate was pushed into the mud at the water's edge, and an area of several square feet of river bed around the plate was shocked with current from a metal hand net electrode held in the surrounding water. Mud banks were sampled by working upstream with at least one assistant downstream to catch any ammocoetes escaping the initial paralysing effect of the shocker. Attempts were made in late July and August to capture large numbers of *fluviatilis* and *planeri* ammocoetes which were showing the earliest stages of metamorphosis (transforming stages). Deeper stony areas and patches of weed and rotting vegetation were also fished when the young transformed lampreys had begun to emerge from the ammocoete 'mud' banks. Animals were transported to the laboratory in either flat dishes or in 'Thermos' flasks.

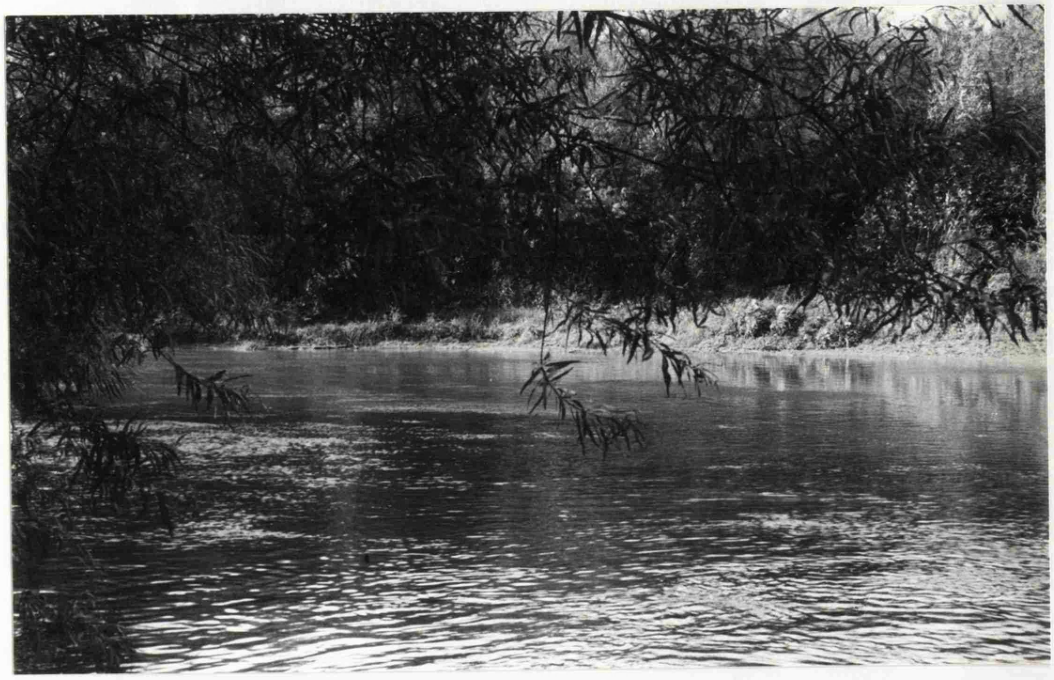


PLATE 1

Ammocoete 'mud' banks at Bransford Bridge, River Teme (Worcestershire) during summer low water conditions.

Larval and metamorphosing stages were measured immediately on return to the laboratory. They were anaesthetised with M.S.222 (Tricain - Methansulphonate, Sandoz), and were measured on millimeter graph paper which had been waterproofed by immersion in molten wax. No animals died during transport or measurement. In the laboratory ammocoetes were kept in large plastic tanks containing aerated river water and a coase sand substrate mixed with river mud. In other tanks metamorphosing stages were supplied with coarse sand, pebbles and horizontally laid tiles. All tanks were kept in a constant temperature room with a daylength of 11 - 13 hours. The temperature was varied to simulate ambient river temperatures (4 - 12^{o}C).

RESULTS

Collections of ammocoetes taken from the Rivers Teme, Taw and Tywi during 1968 and 1969 contained ammocoetes of all three species of British lampreys. *P. marinus* could be separated from *L. fluviatilis* and *L. planeri* by the different caudal shape and pigmentation, (MacDonald, 1958; Vladykov, 1959, 1960). On the basis of the length-frequency distributions of the *P. marinus* ammocoetes, Hardisty (1969) estimated the growth rate and minimum duration of larval life. After removal of *P. marinus* ammocoetes from the collections, diagnostic oocyte counts (Hardisty, 1961) indicated a very high proportion of *fluviatilis* at a few sites. This view was later corroborated by the high proportion of *fluviatilis* in the metamorphosing forms taken from these sites. On the basis of many length-frequency distributions, estimates were made of larval growth rates and the length of larval life of *L. fluviatilis* (Hardisty and Huggins, 1970). A copy of the published estimate is submitted with this thesis.

ii) The capture, salinity tolerance and feeding behaviour of downstream migrants:

By April, transformed *fluviatilis* could no longer be caught by electric fishing in the upper reaches of rivers. However, downstream migrating *fluviatilis* were reported in elver trawls made during high spring tides in March and April near Gloucester on the River Severn. Trawling was carried out in the lowest freshwater reaches of the Severn so that the animals were caught just prior to their entry into estuarine conditions. River temperatures at this time varied between 5°C and 9°C, and all downstream migrants were caught during night-time trawling, although on some nights no lampreys were caught. An otter trawl of fine mesh net was used. The arrival of the high tide in the lower reaches of the Severn reversed the direction of flow. The trawler then took up a midstream station facing into the current, and the trawl was lowered. The midstream station was maintained although on some very high tides the boat and trawl were carried upriver against the full power of the engine. Elvers migrating upriver on the tidal flow were caught and periodically removed from the trawl. As the tide subsided the trawler slowly moved downstream until the river flow returned to normal. Trawling was carried out twice daily on the high tides for about a week. Downstream migrants were transported to the laboratory in the manner described above for ammocoetes.

The 1970 and 1971 elver trawl collections of downstream migrants were measured and their length-frequency distributions were analysed. Proportional body measurements were recorded and means were compared with the same parameters of transformed *fluviatilis* caught and

measured on December 1, 1969 (Hardisty, Potter and Sturge, 1970).

During experiments on their salinity tolerance and feeding behaviour, groups of lampreys were placed into 6, glass-sided, 80ℓ aquaria each containing a 7.5 cm. depth of clean sand and 24ℓ of sea water. Each tank was well aerated and a foam-rubber filter pump was immersed in the water to remove any suspended debris. 2 ceramic tiles (15 cm. × 15 cm.) were placed in each aquarium at an angle of 45^{o} against the sides to provide some areas of light and dark cover on the substrate surface. The aquaria were placed on benches in the constant temperature room and experiments were carried out at temperatures of $4^{o}C$, $6^{o}C$ and $12^{o}C$.

Sea water was at first collected from beaches, but as it varied both in salinity and the degree of pollution, all sea water was subsequently collected offshore by the Marine Biological Association at Plymouth. The salt concentration of this water, as shown by either silver nitrate titration or an Eel Chloride meter, was always between 34.5 and $36‰$.

Four of the six aquaria were set up with full-strength sea water while the other two were filled with sea water diluted by distilled water to concentrations of 33% and 66% of full strength sea water respectively. The pH values of the full-strength and diluted sea water were monitored regularly with a Pye pH meter to check that there was little variation during the experiment. Lampreys were transferred between aquaria by a small hand net.

In April 1970, two groups of lampreys were transferred directly from fresh to full-strength sea water. One group consisted

of 25 animals caught in the Rivers Teme, Taw and Tywi during the autumn and winter of 1969, and subsequently maintained in freshwater aquaria in the constant temperature room. The other group of 21 animals had been caught as downstream migrants by elver trawling in April 1970.

In an acclimation experiment, 18 downstream migrants, also caught by elver trawling in April 1970, were placed for five days in first 33% sea water and then 66% before being transferred to full-strength sea water. In all experiments records were kept of the numbers dying and the date of deaths during 28 days in full-strength sea water. Records of the behaviour of 10 animals undergoing acclimation were kept, with particular reference to whether they were a) burrowed in the substrate, b) lying under ceramic tiles, c) lying under glass tiles or d) lying uncovered on the substrate surface.

During salinity tolerance experiments, small fish were released into aquaria and inspected daily for wounds caused by lamprey attacks. Species of fish offered as prey were Bass *(Dicentrarchus labrax)*, Thick-lipped grey mullet *(Crenimugil labrosus)*, Flounder *(Platichthys flesus)*, Sea snail *(Liparis liparis)*, Three beared rockling *(Gaidropsarus vulgaris)*, Poor Cod *(Trisopterus minutus)*, and Rainbow trout *(Salmo gairdneri irideus)*. The fish were 5 - 15 cm. long and were obtained from the Severn estuary, the Marine Biological Association at Plymouth and a local trout hatchery.

The behaviour of some lampreys following the presentation of fish was examined in detail. The behaviour of lampreys in aquaria was

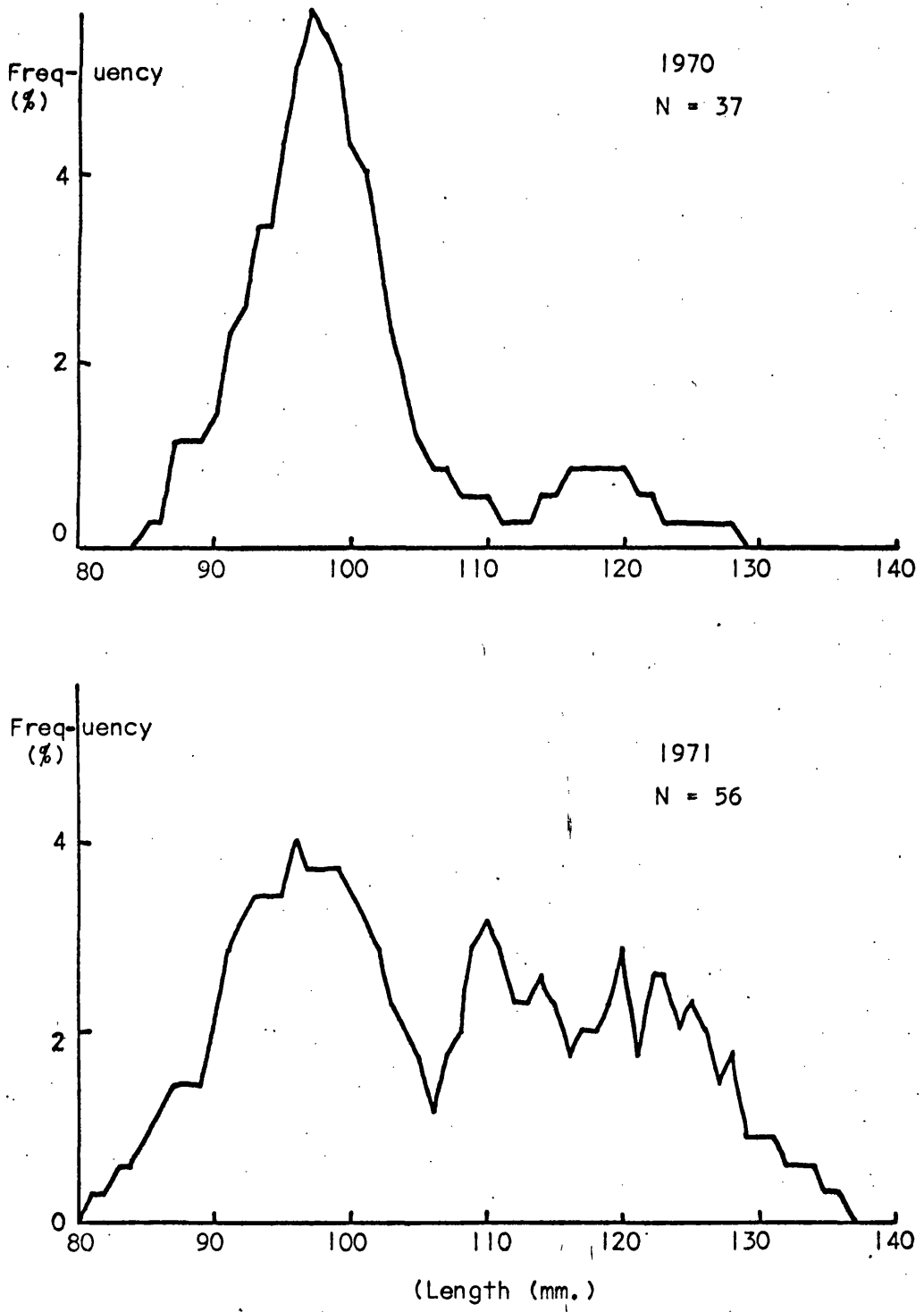
observed for 15 min, after which a live fish was introduced into one aquarium and a freshly killed and lacerated fish, suspended on a wire support, was immersed in another. After the fish had been presented, the behaviour of the lampreys was examined for a further 15 min. If, after this time, lampreys had not attached to the dead fish, it was rapidly moved, using the wire support so that it was constantly in close proximity to a lamprey in an attempt to induce attachment. The behaviour of any lampreys which attached was observed and recorded. Attempts to dislodge attached lampreys by vigorously shaking the dead fish, permitted an assessment of the strength of attachment.

RESULTS

Elver trawling in 1970 and 1971 during April resulted in the capture of 39 and 56 downstream migrants respectively. Length frequency distributions of the two collections (Figure 1) show at least one well-defined mode with a mean in both years at 98 mm. The factors which may be responsible for modes within these populations will be discussed later.

A comparison of the proportional body measurements of spring-caught downstream migrating *fluviatilis* with those of animals electro-fished from rivers during the winter showed only two significant changes. Both the length of the branchial region and the depth of the body significantly decreased (Table 1). The second of these changes is probably due to the period of starvation between the onset of metamorphosis in early autumn, and the beginning of estuarine or marine feeding in late spring.

FIGURE 1



Length-frequency curves for downstream-migrant *L. fluviatilis* caught in elver trawls in the River Severn during April 1970 and March 1971.

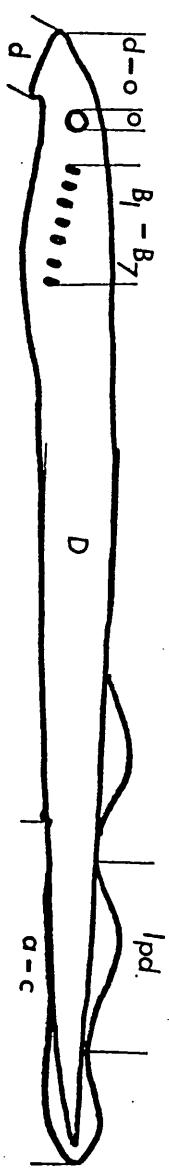
TABLE 1

Lengths and body proportions (mean ± 1 standard error of mean) for metamorphosing
Sturge, 1970) and downstream migrating *L. fluviatilis*

	T L	$\frac{d}{T L}$	$\frac{d\text{-}o}{T L}$	$\frac{o}{T L}$	$\frac{B_1\text{-}B_7}{T L}$	N
Metamorphosing I DECEMBER 1969 (From Hardisty, Potter and Sturge, 1971)	100.3 ± 1.31	4.90 ± 0.11	7.7 ± 0.13	2.7 ± 0.14	9.8 ± 0.049	45
Downstream migrants caught by elver trawling in APRIL 1970	99.99 ± 1.37	5.13 ± 0.07	7.88 ± 0.08	2.84 ± 0.05	9.05 ± 0.06	37
					p<0.001	

Body proportions are expressed as a percentage of the total length.

a-c	tail length	D	depth of body
B_1-B_7	length of branchial region	Lpd	length of posterior
d	disc length	o	diameter of eye
d-o	length of pre-orbital region	N	number of animals



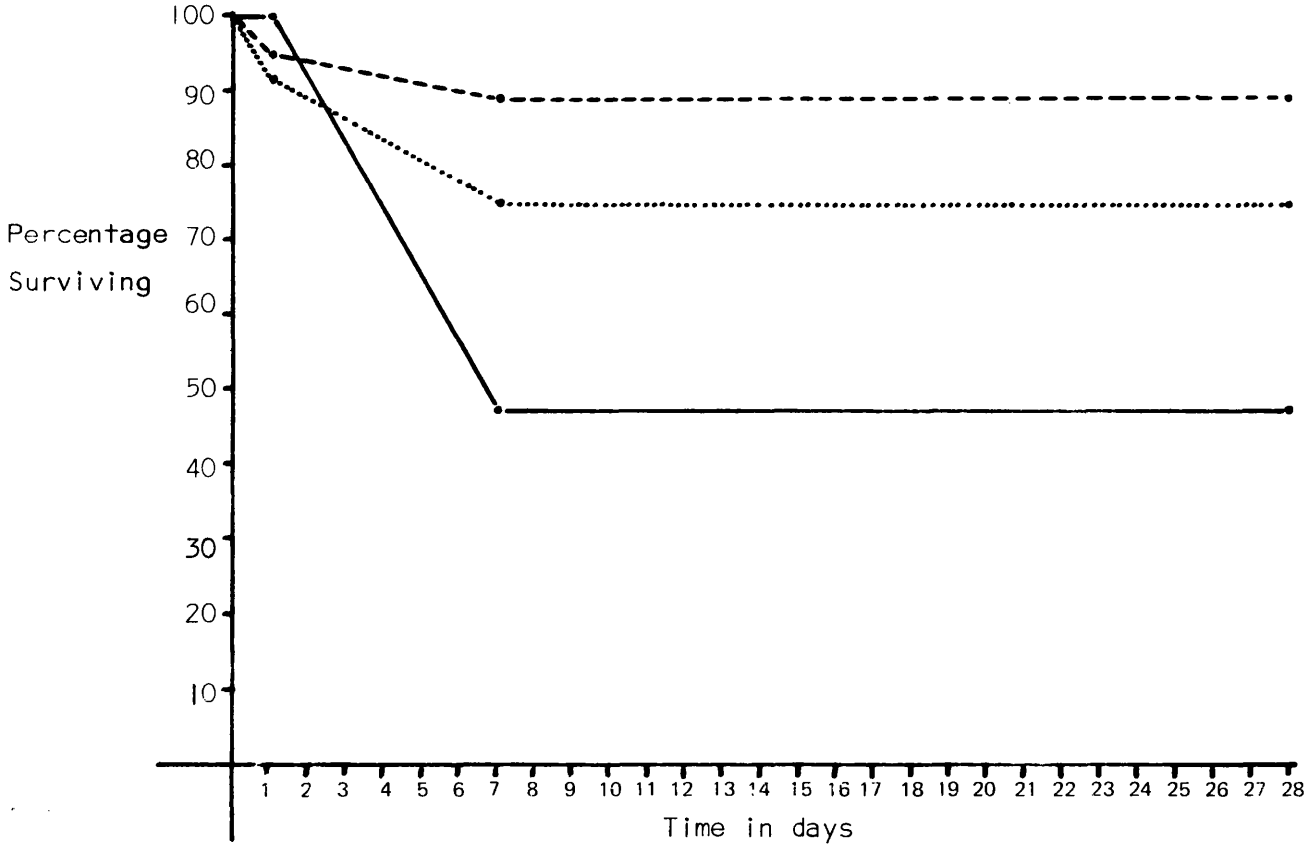
Of the three groups of lampreys placed in full-strength sea water, the highest percentage survival after 28 days was shown by recently caught animals which had been acclimated before transfer, while the lowest was shown by non-acclimated lampreys which had been maintained in the laboratory over winter (Figure 2).

Some lampreys in full-strength sea water developed swollen abdomens (Plate 2). 8 animals with this condition were killed and sectioned and the resulting slides (Plate 3) demonstrated that the swelling was due to the presence of water in the alimentary canal. Some lampreys exhibited the swollen abdomen condition and died following direct transfer, while others developed the condition and then recovered. Approximately one third of the lampreys transferred to full-strength sea water showed some degree of abdominal swelling.

The daytime behaviour of 10 lampreys in 33% and 66% and full-strength sea water is shown in Figure 3. Prior to the experiment, 6 of the 10 lampreys in fresh water had remained burrowed during the day. Transfer from fresh water to 33% sea water evoked daytime emergence from the substrate and further emergence occurred after transfer from 33% to 66%. Most of the emerged animals were found lying under ceramic tiles. Transfer from 66% to full-strength sea water resulted in the death of two lampreys, and after 5 days in full-strength, all the remaining lampreys had emerged from the substrate.

Only one lamprey attack on a live fish was observed. A small flounder had been placed in an aquarium, and within a few second swam over a lamprey which was lying on the substrate surface. The lamprey swam rapidly under the flounder and immediately attached ventrally just

FIGURE 2



Percentage survival of 3 groups of recently transformed River lampreys directly transferred to full strength seawater in Spring 1970.

Solid line —— 25 non-acclimated lampreys held in laboratory since beginning of metamorphosis.
Dotted line....21 non-acclimated recently caught lampreys.
Dashed line----18 acclimated recently caught lampreys.

Experiments at 8-12°C. 11-13 hr daylength.

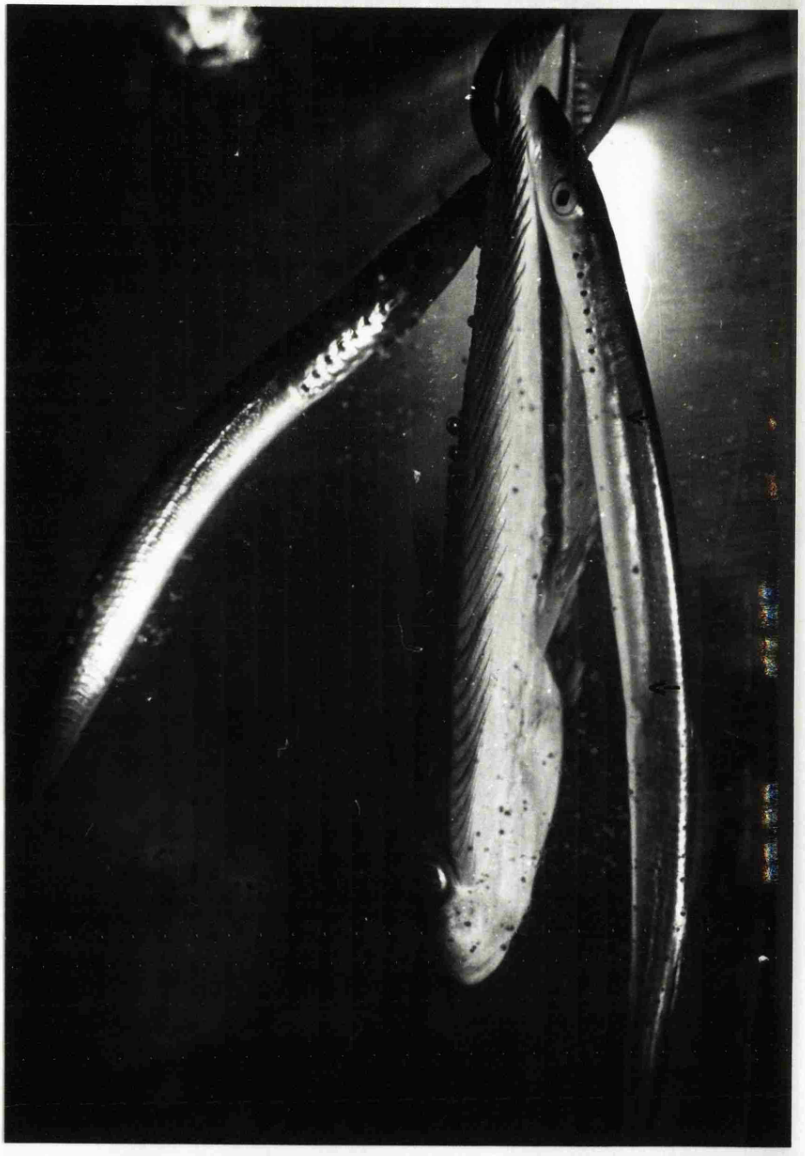


PLATE 2

Transformed river lamprey in full-strength sea water attached to a dead flounder and showing a swollen abdomen condition. (Extent of swelling marked by arrows) April 1970.

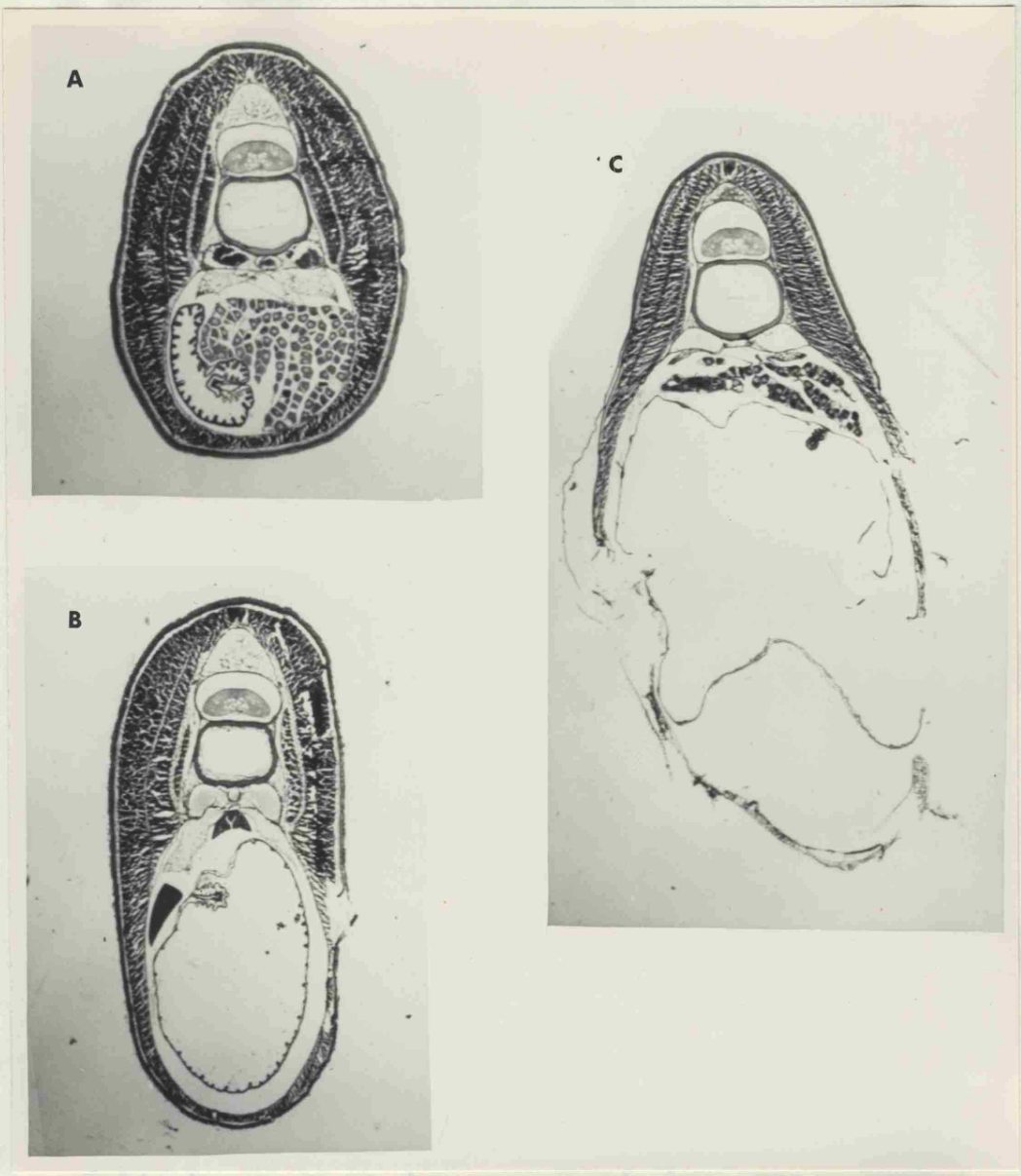


PLATE 3

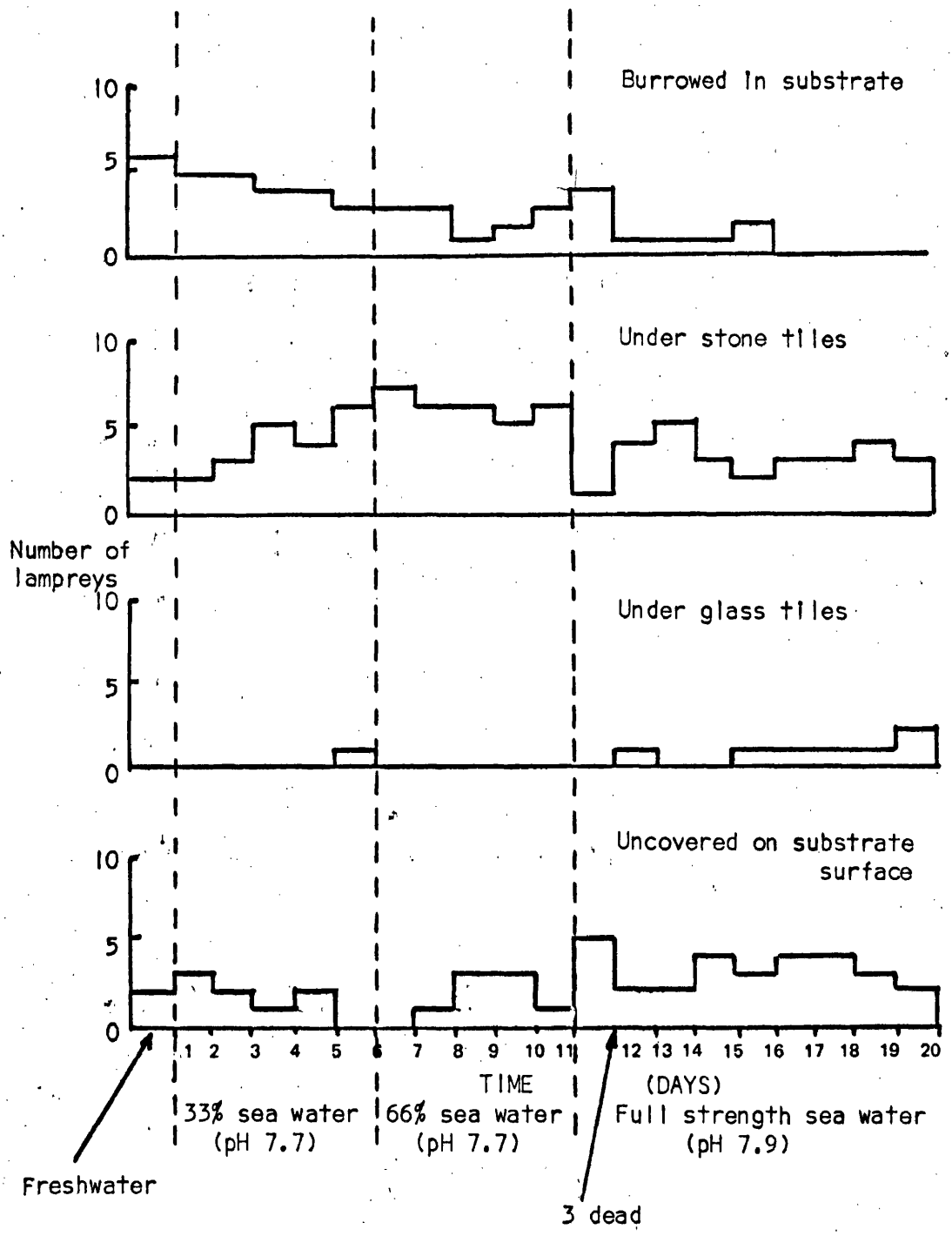
Sections of 3 transformed *fluviatilis* which had been transferred to full-strength sea water.

A. Normal, no abdominal swelling.

B. Partial abdominal swelling, alimentary canal distended by water.

C. Extreme abdominal swelling. Animal embedded and sectioned just after death. (Grossly distended body wall broken up during sectioning).

FIGURE 3



Effects of increased salinity on the behaviour of 10 transformed River lampreys in April 1970 (5°C)

behind the operculum. The flounder then began vigorous swimming, and appeared to make attempts to dislodge the lamprey by scraping it on the substrate surface. The lamprey was not dislodged, and both animals sank onto the substrate where they lay motionless for some minutes. Only the tail of the lamprey was visible during this period. The flounder then swam off, and the lamprey, which had detached, was left lying on the substrate where it remained for the rest of the observation period. The flounder was inspected but no epidermal damage was evident as a result of the attack. Although this was the only witnessed attack on a live fish, the skin of several fish exhibited slight lesions, consisting of circular abrasions of similar diameter to that of the lamprey sucker. If the slight abrasions were due to lamprey attacks, these must have occurred during the dark period of the daily cycle when no observations were made.

The introduction of live or dead fish into aquaria containing lampreys in early April at $4^{o}C$ and $6^{o}C$ evoked no observed changes in the behaviour of the lampreys. At $12^{o}C$ in late April, May and June, two marked behavioural patterns were observed following the introduction of fish.

1) The presence of either live or dead fish in an aquarium sometimes resulted in vigorous head-shaking. These movements occurred when lampreys were swimming and were often associated with a backwards motion of the whole animal. Similar head-shaking movements were frequently observed when lampreys were first placed in full-strength sea water. During behavioural studies of they prey-catching activity of Sea lampreys, Groot (1958) also described head-shaking movements, but was unable to explain them.

2) Some lampreys exhibited 'slow-swimming' following the introduction of fish into the aquarium. Characteristically, the sucker of a 'slow-swimming' lamprey was cup-shaped, and was held higher than the rest of the body. This type of swimming was presumably equivalent to 'oblique' swimming as observed and described in the behaviour of feeding *P. marinus* by Lennon (1954) and Groot (1958).

The presence of dead and lacerated fish evoked the most prolonged slow swimming, and lampreys often swam towards a dead fish against the water current produced in the aquarium by the air-bubble stream of the aerator. After passing close to a dead fish in the water current, lampreys frequently attached to the aquarium wall, and proceeded to rasp at the glass surface. The strongest rasping movements were associated with a 'flick' in the body and tail, presumably equivalent to the 'beats' described in the feeding behaviour of *P. marinus* by Groot (1958). The flicks often became so vigorous that the animal appeared to lose momentarily its attachment to the glass, thus executing a series of short 'jumps' resulting in a shift of the point of attachment.

After induced attachment to a dead fish, lampreys could not easily be detached, even by very vigorous shaking. They executed the flicks and jumps previously described, and often left a path of slight epidermal damage on the flounder (Plate 4). One induced attachment to the left mid-dorsal region of a small dead Rainbow trout resulted in a circular lesion, 4 mm in diameter and 3 mm in depth, which exposed subcutaneous muscle tissue. Periods of induced attachment varied from 2 minutes to several hours.

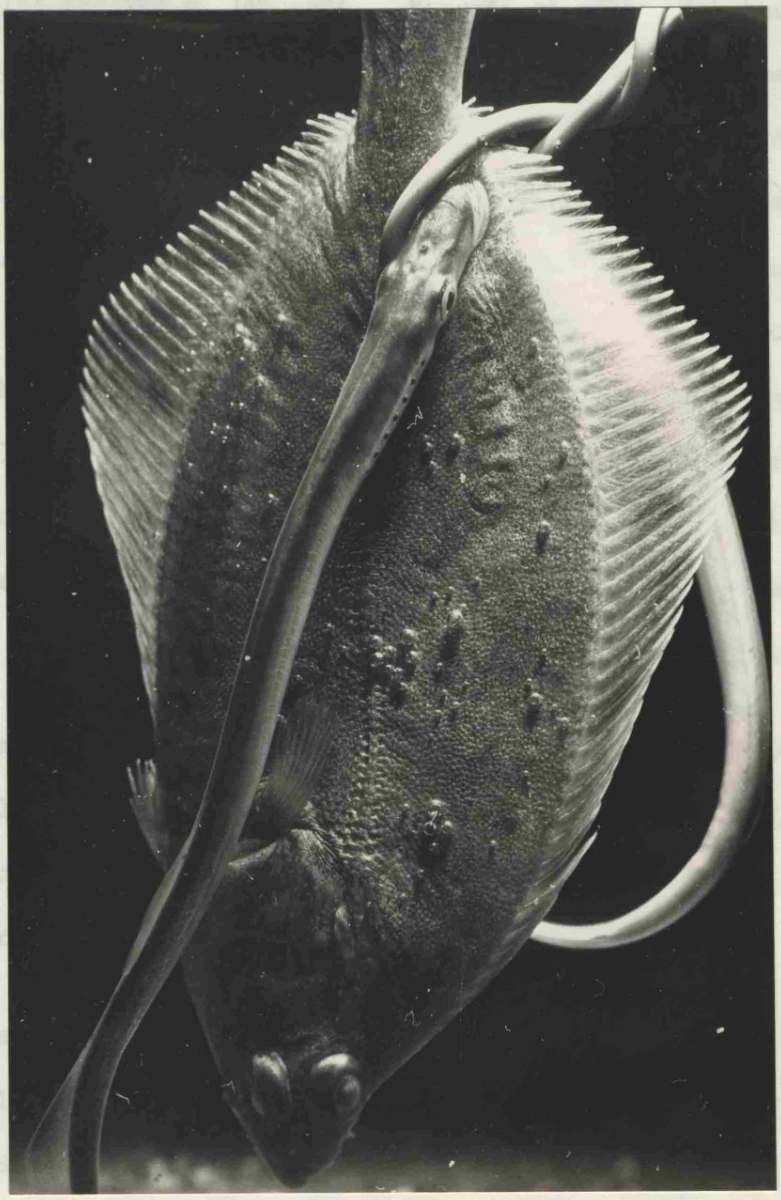


PLATE 4

Dead Flounder in sea water to which a young *L. fluviatilis* has been induced to attach. Arrows show epidermal damage which has been caused by the lamprey.

Much colourful local information was collected on the marine and freshwater feeding of lampreys. Reports of trout with "hundreds of small lampreys attached to them" were received from fishermen on the upper reaches of the River Severn and a "huge" lamprey was said to have attacked the legs of horses as they forded the River Tywi in Carmarthenshire. Apart from the entertaining myth and folklore, some authentic and useful information was collected. The Plymouth Marine Fauna (3rd Ed., 1957, M.B.A.), records two lampreys caught attached to fish at sea. The first, listed as a "juvenile", 80 mm in length was caught attached to a mackerel *(Scomber scombrus)* off Plymouth on 13.1.1950. The second record refers to a Sea lamprey, 22.5 cm long, which was attached to a Grey Mullet *(Mugil labrosus)* caught in Millbay Dock, Plymouth on 6.1.1953.

Members of Bath University Sub-Aqua Club reported "10 to 20 slate-grey lampreys" attached to the ventral surface of basking sharks off the Cornish coast, and Maxwell (1952) records instances of captured basking sharks being "infested" with Sea lampreys.

Salmon and Sea trout bearing lamprey marks have been recorded, and especially authentic are the reports by coracle fishermen who net salmon and Sea trout in the tidal reaches of the River Tywi. Water bailiffs at Shrewsbury on the Severn have caught salmon with fresh lamprey wounds, and have described, in some detail, the 'pin-pricks' within the wound presumably caused by the lamprey's teeth.

A freshwater feeding form of *L. fluviatilis* has been reported from Loch Lomond (Robertson, 1875; Maitland, pers. comm.), and in 1971 a visit was made to the Glasgow University Field Station at Rowardennan on Loch Lomond, to collect further information. As a result of gill

and seine netting in the loch, Brown trout and Powan *(Coregonus spps.)* were caught bearing healed lamprey wounds. In some catches 30% of the Powan displayed at least one lamprey scar, and fish with 2 or 3 scars were common. In view of the paucity of documented information on the feeding phase of *L. fluviatilis*, due mainly to the inaccessibility and dispersion of marine populations, this interesting landlocked population deserves further attention.

iii) Factors affecting the capture of upstream migrants:

In recent years, large numbers of live anadromous River lampreys have been supplied, primarily in October and November by Mr. P. Gaskins of Haw Bridge, near Tewkesbury, Gloucester. The method of collecting is simple and effective. Lampreys are apparently able to migrate upstream only as far as the weir at Severn Ham near Tewkesbury, which they cannot easily pass unless a tide of sufficient height (at least 8.5 m. at Sharpness Docks), raises the water level downstream of the weir. On suitable tides, a tubular wire mesh trap, about four feet long, is lowered just under the surface of the water race at the side of the weir, with the funnel of the trap facing downstream. Lampreys attempting to pass the weir at the side are caught in the trap. Fishing is restricted to the hours of darkness, since little, if any, daytime movement of lampreys takes place.

Lampreys were also collected from the 'trash' baskets of nuclear power stations on the Severn estuary at Berkley and Oldbury-on-Severn. Lampreys from this source, when considered with the catches made at Tewkesbury, provided data on the timing of the migration, as the Power stations are located in an estuarine environment, while Tewkesbury samples are from freshwater. Both Power stations use complex filter systems to remove debris and small animals from the cooling water inflow. River lampreys were found in the trash baskets during the early autumn of 1969 and in subsequent years, although many were dead or damaged as a result of their passage through the filtration system.

Captured River lampreys were transported to the laboratory in

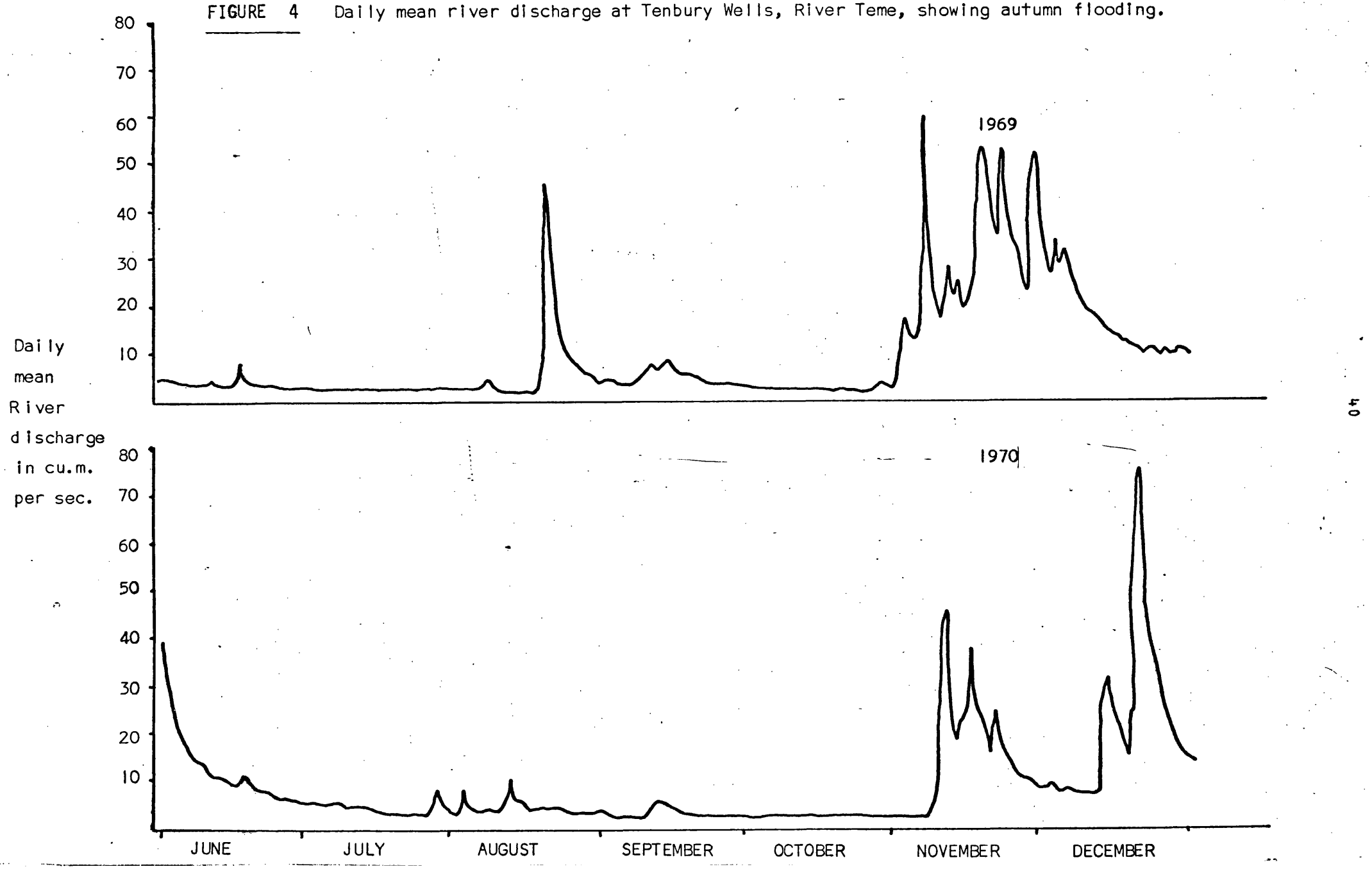
large plastic containers, half filled with river water. None of the animals from Tewkesbury died during transportation, but 30% mortality sometimes occurred during the transport of animals from the Severn estuary Power stations. At the laboratory in 1969, River lampreys were kept in wire mesh cages submerged in a concrete-sided aerated pond. The wire cages appeared to cause skin lesions on some lampreys, and in 1970 and 1971 the animals were released into the pond and caught with a hand net when required.

RESULTS

Anadromous River lampreys are normally available at Tewkesbury in late October and November, and the biggest catches are usually made in late November. In some years, lampreys may be caught at Tewkesbury as early as August or September, but in 1970 and 1971 there were no such early migrants. At Oldbury, large numbers of lampreys appeared in the trash baskets during late October in 1969 and 1970.

From his own observations, Mr. Gaskins has suggested that increased downstream flow of freshwater is necessary to stimulate activity in upstream migrants. With the aid of data supplied by the Severn River Authority, the river flow (expressed as daily mean discharge in cubic metres per second), during the summer and autumn months, was calculated for the River Teme and the graphs for 1969 and 1970 are shown in Figure 4. The river flow was recorded at Tenbury Wells on the River Teme, a major tributary of the River Severn. The drainage area of the gauging station at Tenbury is only 1130 km^2, and the Severn in its lower reaches may exhibit autumn flow rates even more pronounced then those recorded from the Teme at Tenbury.

FIGURE 4 Daily mean river discharge at Tenbury Wells, River Teme, showing autumn flooding.



The sudden and violent flooding recorded in both years during October and November supports a correlation between increased downstream flow and upstream migration of River lampreys. The minor floods which occasionally occur in August or September of some years may likewise be responsible for early migrants which sometimes appear.

iv) Spawning conditions and sites:

Since no information was available on the location of River lamprey spawning sites in Britain, the rivers Severn, Teme and Tywi, in which large ammocoete populations of *L. fluviatilis* occurred, were surveyed for likely spawning areas. European temperature records for spawning, and mean monthly river temperatures supplied by River authorities, indicated that the approximate breeding time in south west Britain was likely to be March and April. Observation of likely sites during these months led to the initial capture of spawning animals from all three rivers in 1969.

RESULTS

Only one major *L. fluviatilis* spawning site was discovered on the River Severn. At Shrewsbury, the river flows over a large weir, and approximately 200 m. downstream it passes over a shingle bed at a depth of 35 - 100 cm. Numerous empty redds were found amongst the shingle in late April 1969, and a few spent lampreys were collected. Most of the redds were on the outer edge of the shingle bank where the water was 70 - 90 cm. deep. The redds were irregularly shaped although some of the smaller redds were almost circular and saucer-shaped. During 1970, the weir at Shrewsbury fell into some direpair, and no lampreys spawned on the shingle bank. It was assumed that immature upstream migrants were able to pass the damaged weir. No visits were made to the site in 1971.

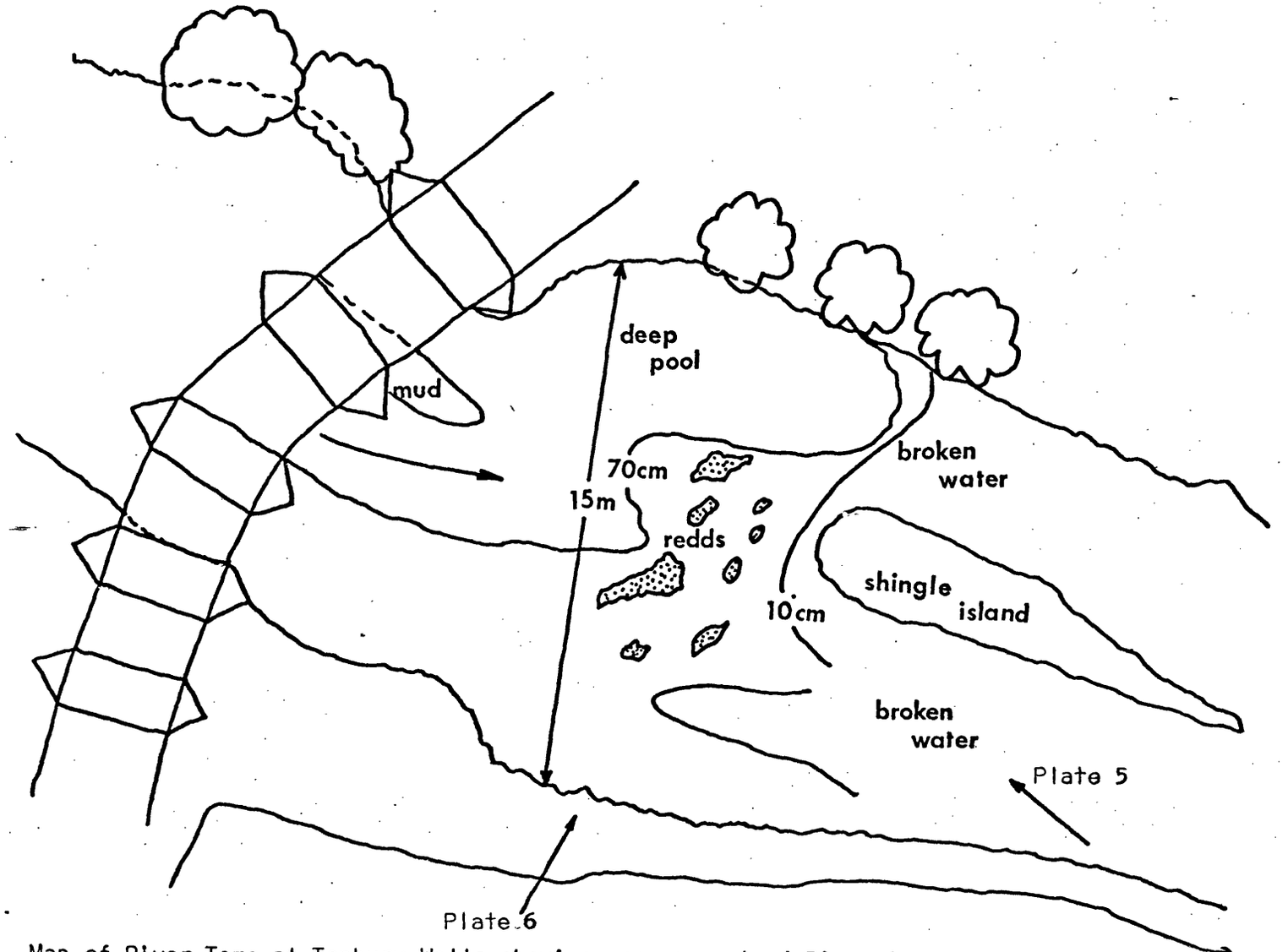
On the River Teme, spawning *L. fluviatilis* have been observed

and collected from several sites between Tenbury Wells and Ashford Carbonnel. At one site in Tenbury (Figure 5 and Plates 5 and 6), the Teme flows under a road bridge and into a deep pool before 'breaking' over an extensive shingle bank, which causes much rippling and broken water. At this site, redds were found each year in the same general position, their arrangement in 1971 being shown on the map (Figure 5). Redds were found in water 22 - 52 cm. deep, and most were irregularly shaped. Many of the larger redds were extensive excavations, and the exposure of large irregularly shaped rocks, underlying the shingle, determined the redd shape. Most redds were 10 - 15 cm. deep, and excavated shingle was deposited in a stone pile immediately downstream of the redd. Some stone piles extended 30 cm. from the downstream edge of the redd.

Approximately 750 m. upstream from this major spawning site in Tenbury, the Teme flows under a high bank on one side, on which is sited a churchyard and from which, over a period of years, rocks, masonry and large stones have been thrown into the river, forming an artificial bank of shingle and stones. This stone bank now extends across the full width of the river, and lying on the river bed are some large stone slabs under which immature, ripe and spent River lampreys were found, especially large collections being characteristic of the 1971 spawning run. Extensive spawning occurred over the stone bank, but because of the nature of the river bed, the redds were irregular in shape, and in some cases, redds were found which extended under the large stone slabs.

At Ashford Carbonnel, about 5 km. upstream from Tenbury Wells, the Teme passes over a high weir which River lampreys, migrating

FIGURE 5



Map of River Teme at Tenbury Wells showing arrangement of River lamprey redds during spawning period in 1971

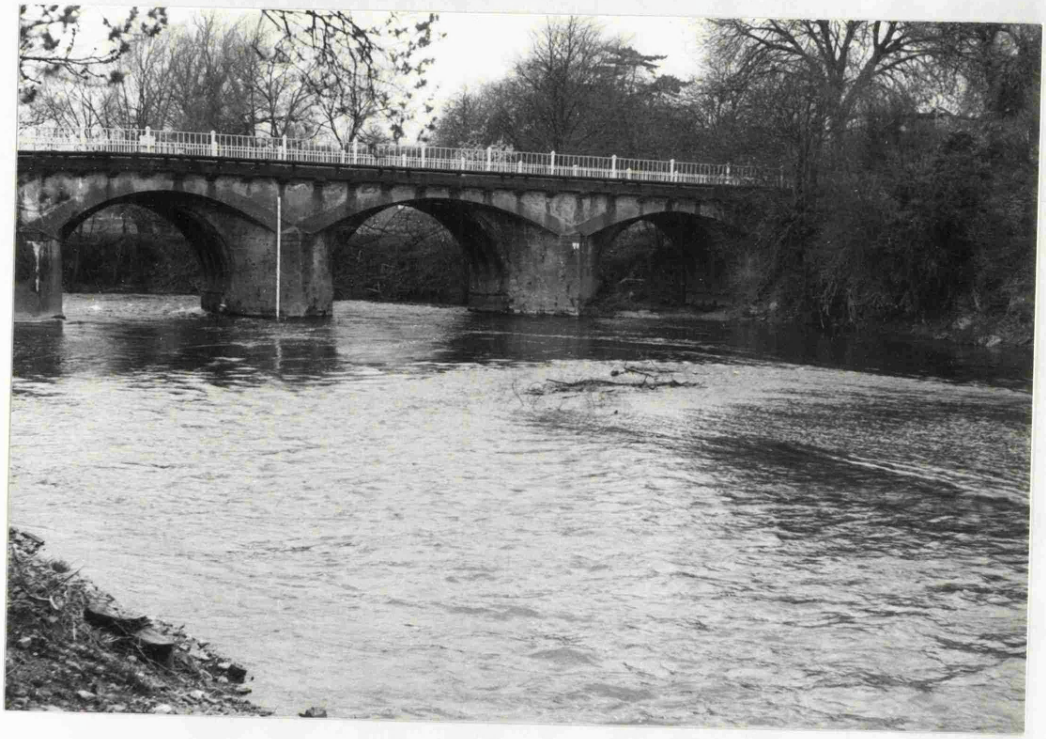


PLATE 5

River lamprey spawning site at Tenbury Wells on the River Teme.

A map of this site is shown in Figure 5.

PLATE 6

Another view of the River lamprey spawning site shown in Plate 5. The camera directions of these two photographs are indicated on Figure 5. .

upstream, apparently cannot surmount, since many immature animals were found concealed under large flat stones lying on the bed of the weir pool. Immediately below the weir, the river flows over several extensive shingle beds, and many redds were discovered in this region. Redds contained spawning groups of between 3 and 23 lampreys. Most redds were in mid-river, and none were found in the extensive shaded areas caused by large overhanging trees. At this site and the others on the Teme, small numbers of *L. planeri* were found in or near redds constructed by *L. fluviatilis*.

In contrast to the rivers Teme and Severn, which in their middle reaches mainly run deep and only occasionally break over shingle banks, the River Tywi is a wide shallow river for most of its length, and in its middle and upper reaches, runs over a substrate of almost exclusively shingle, shale and large pebbles. In April 1969, local observers reported that River lampreys were spawning in side streams and in one small tributary of the Tywi, the Afon Marlais, near Llangadog, *L. fluviatilis* and *L. planeri* were found spawning together, observations of which have already been published (see enclosed reprint; Huggins and Thompson, 1970). Part of the Afon Marlais is shown in Plate 7, and redds were found under the left hand edge of the small bridge. No spawning *L. fluviatilis* were seen during further observations of the side stream in 1970 and 1971, although numerous spawning *L. planeri* were found. Spawning River lampreys were caught from the main river during 1970 and 1971. In both years small isolated *L. fluviatilis* redds were found over a 2.5 km. stretch of the Tywi in the Llangadog region, and 7 *L. fluviatilis* were caught. All were caught from redds which also contained spawning *L. planeri*. Spawning *L. fluviatilis* were observed

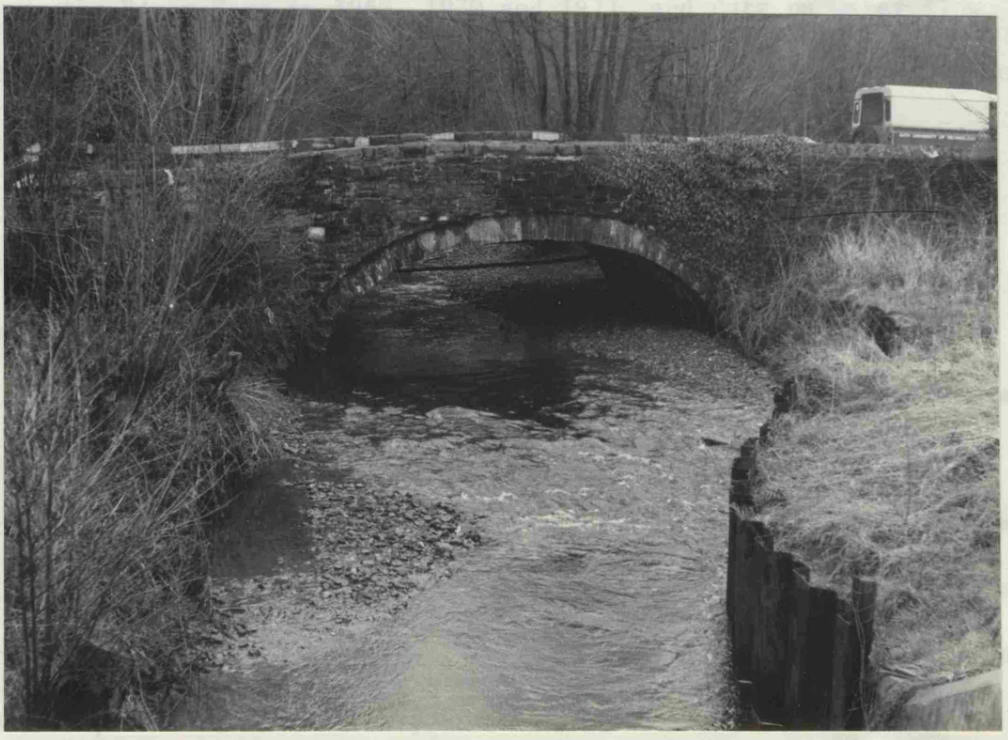


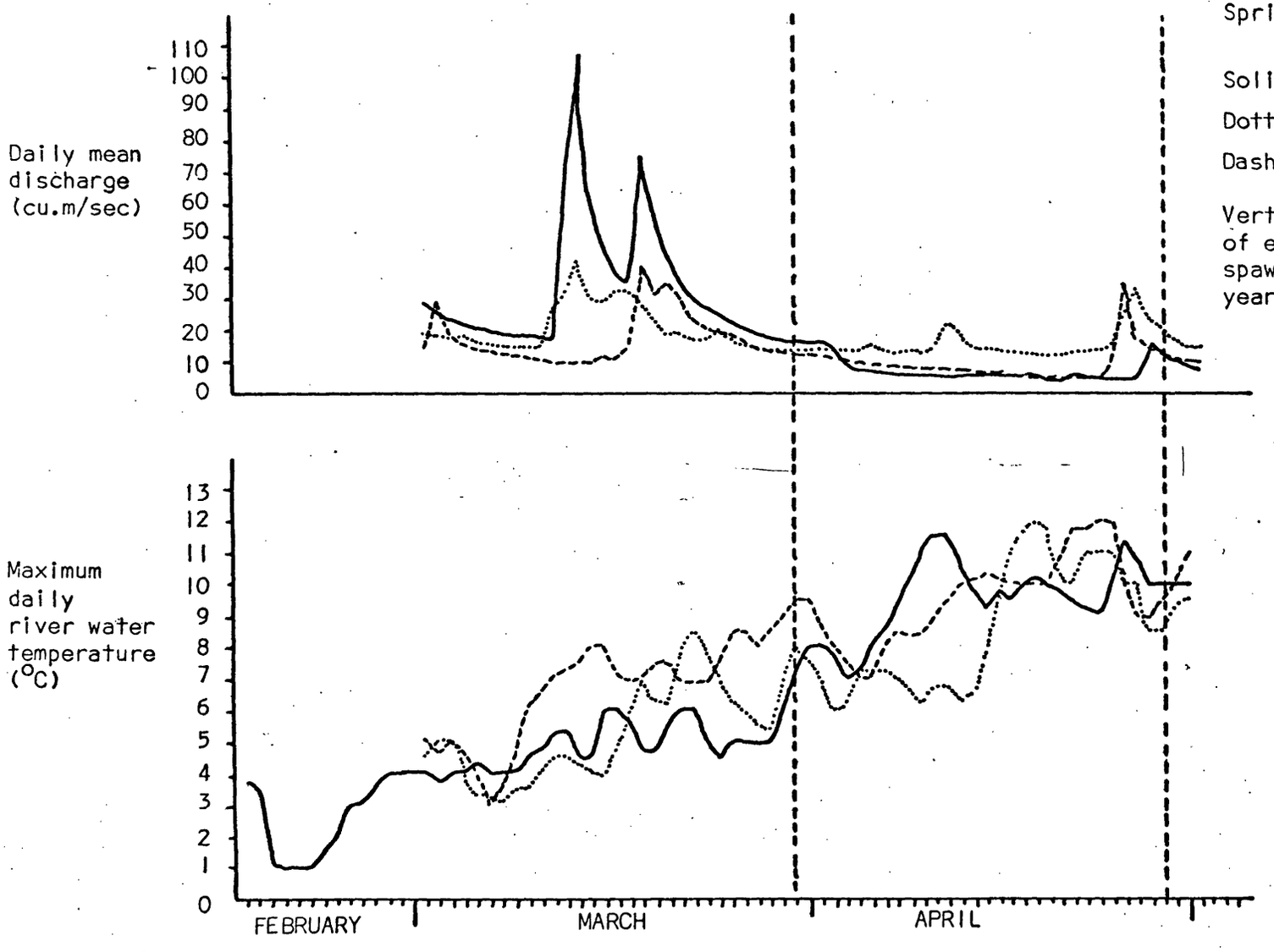
PLATE 7

The Afon Marlais, a tributary of the River Tywi. This photograph shows the site of the River lamprey redd described in the text and in Huggins and Thompson (1970).

in the Tywi on the 27th April and 8th May, 1969; the 6th May, 1970 and the 22nd April, 1971.

Since only a few spawning *L. fluviatilis* were captured from the River Tywi, it was difficult to correlate spawning with river conditions. However, many spawning *L. fluviatilis* were collected from the River Teme in 1969, 1970 and 1971, and data on water flow and river temperatures in these years were available from the Severn River Authority (Figure 6). Spawning in the Teme occurred over a period of approximately one month in all three years, with the 30th March, 1971 being the earliest date on which redd construction was observed. The latest date in the three years on which spent lampreys were caught was 28th April, 1969. These data indicate that spawning occurred during a period of continuously rising water temperature associated with a period of comparatively low water flow in all three years (Figure 6). River lampreys were found in or near redds over a wide range of river temperatures (6 - 12.5^{o}C). Little activity was observed at temperatures below 8^{o}C, but at higher temperatures, redd building frequently took place although the shedding of eggs and milt seldom occurred at temperatures below 10.5^{o}C.

FIGURE 6



River flow and temperature at Tenbury Wells, River Teme during Spring 1969, 1970 and 1971

Solid line —— 1969

Dotted line 1970

Dashed line ---- 1971

Vertical dashed lines show the dates of earliest and latest capture of spawning lampreys during the three years.

Morphometric studies of spent *L. fluviatilis*

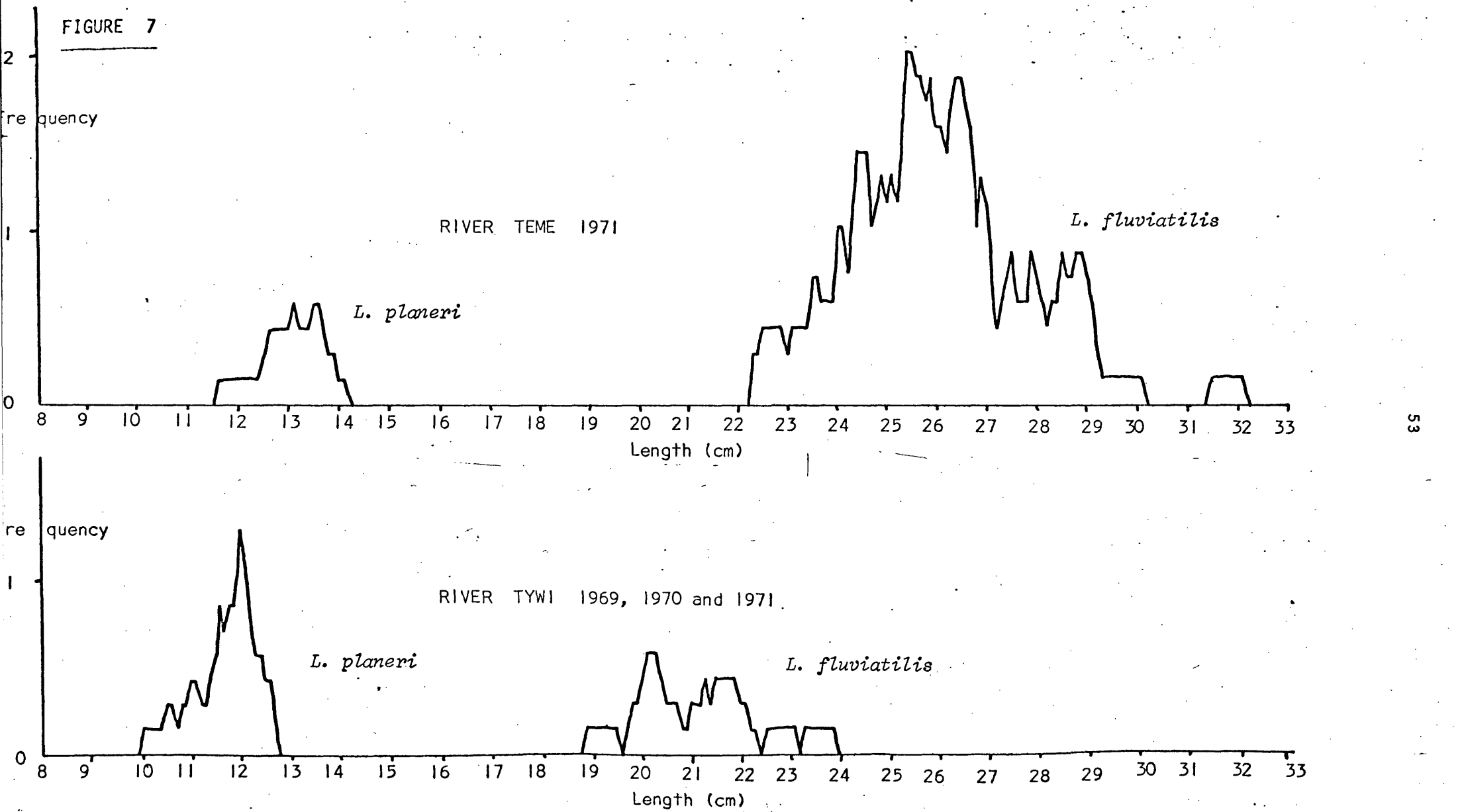
Since sexually-maturing River lampreys undergo considerable shortening during their anadromous migration (Larsen, 1962), it is difficult to compare sizes of River lampreys from difficult rivers at different stages on their migrations. Spent River lampreys, immediately after spawning may be more comparable, since these animals will have fully undergone allometric reductions in length and weight.

Spent *L. fluviatilis* and *L. planeri* were captured in 1969, 1970 and 1971 from the River Tywi, and in 1971 from the River Teme. The spent populations were composed of 13 *L. planeri* and 9 *L. fluviatilis* from the Tywi, and 8 *L. planeri* and 70 *L. fluviatilis* from the Teme (Figure 7 and Table 2). No significant differences were found between the mean lengths of spent male and female *L. fluviatilis* from the same river, but comparison of the mean length of Tywi males with that of Teme males showed a highly significant different ($p < 0.001$) and the mean length of Tywi females was also highly significantly different from that of Teme females ($p < 0.001$) (Table 2). Mean weights of Teme animals were more than double those of Tywi animals.

During elver trawling at Gloucester in March and April of of 1970 and 1971, 5 unusual River lampreys were caught, 3 in 1970 and 2 in 1971 (Table 3). One of the animals caught in 1970 was photographed (Plate 8) together with a downstream migrant *L. fluviatilis* and a spawning-run adult caught from the upper reaches of the Teme. The animal was dissected and found to be a female with a large intestine and well developed eggs. It possessed fewer eggs than lampreys caught during the autumn spawning migration. Small lampreys of this

type have occasionally been caught at Tewkesbury during the spring, and they characteristically are of small size, with a large gut and small egg number (Hardisty, pers. comm.). The smaller animal caught in 1970 was placed in full-strength sea water, and survived for 22 days before dying of unknown cause.

FIGURE 7



Length-frequency distributions of spent *L. planeri* and *L. fluviatilis* caught in 1969, 1970 and 1971 from River Tywi, and in 1971 from the River Teme. Data smoothed by running average of 7mm.

TABLE 2

Mean lengths and weights (± standard error of mean) of spent River lampreys from the rivers Teme and Tywi. (N = number of animals).

Source and sex of spent *L. fluviatilis*	N	Mean length in cm.	Mean weight in gm.
River Tywi males	5	20.4 ± 0.4	16.2 ± 0.8
River Tywi females	4	21.3 ± 0.7	16.9 ± 0.5
River Teme males	27	25.8 ± 0.4	38.2 ± 1.7
River Teme females	43	26.1 ± 0.3	40.7 ± 1.2

TABLE 3

Adult River lampreys caught during elver trawling for downstream migrating River lampreys at Gloucester, River Severn.

* Indicates animal shown in Plate 8.

Date of capture	Length in cm.	Weight in gm.
April 1970	21.4	13.3
	19.1	10.5*
	22.4	18.4
April 1971	24.5	10.3
	30.2	24.3

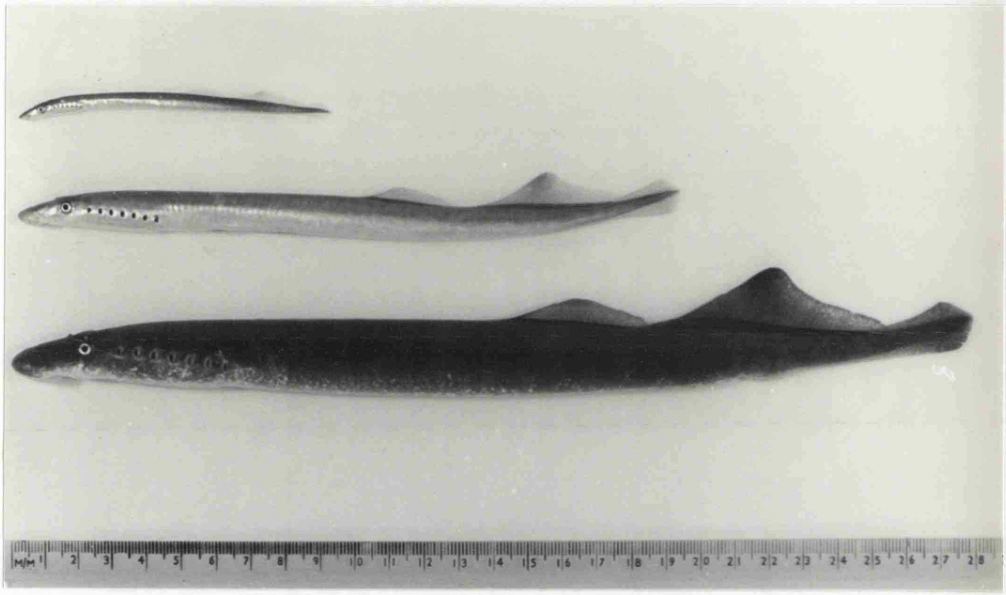


PLATE 8

Postmetamorphic stages of *L. fluviatilis* caught in the River Severn during April 1970.

A. Downstream migrant.

B. Spring upstream migrant. (see Table 3)

A and B were caught during elver trawling in the lower reaches of the Severn.

C. Autumn upstream migrant caught from a spawning region in the upper reaches of a Severn tributary (River Teme).

C. DISCUSSION

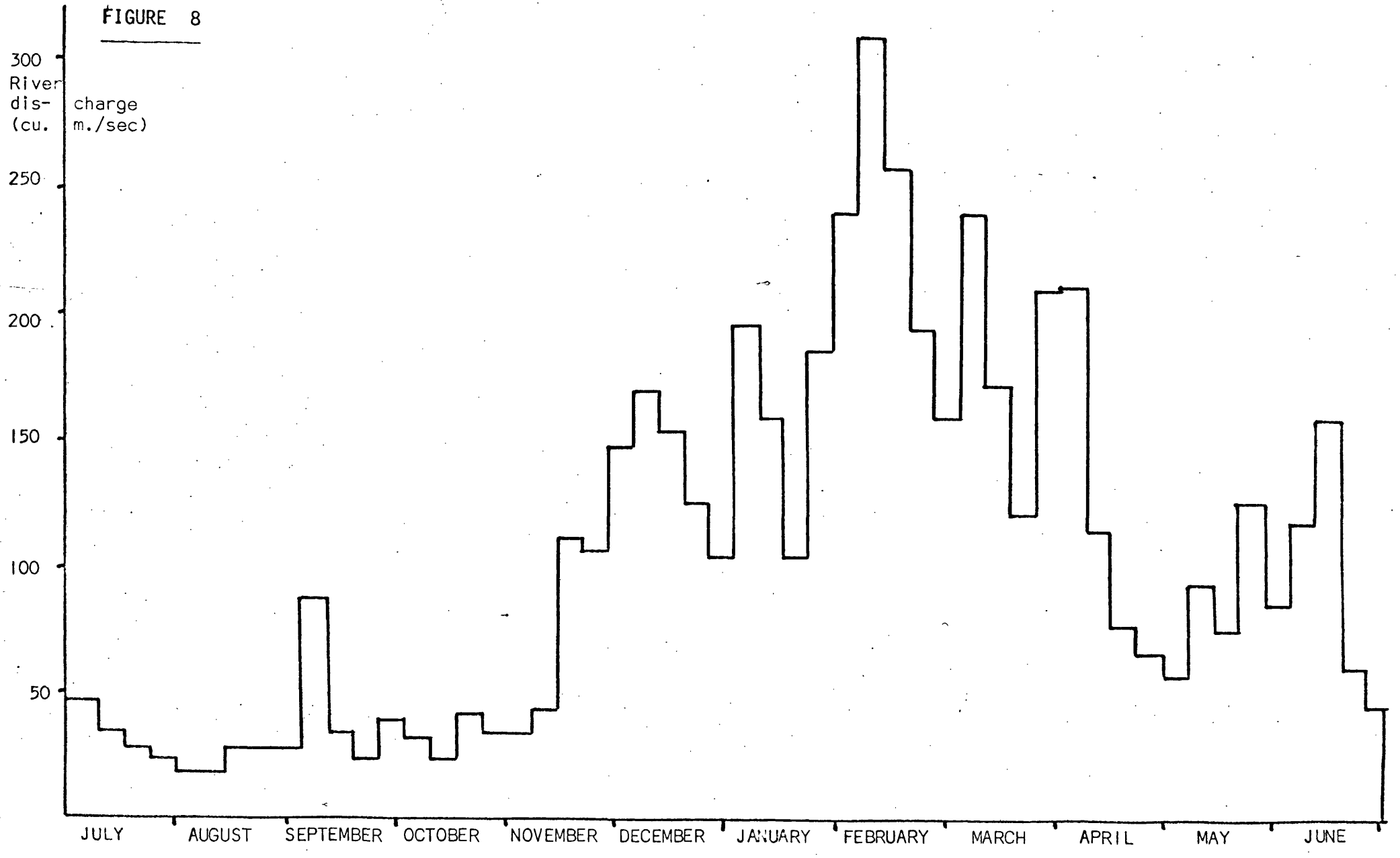
The duration of the larval period of *L. fluviatilis* in the rivers investigated was estimated as four and a half years (Hardisty and Huggins, 1970). The length-frequency distributions on which these estimates were based resemble those recorded for populations of the same species in the rivers of the Gulf of Riga by Privolnyev (1964), in that three well-defined modes are present. Privolnyev's collections were made immediately after the onset of metamorphosis, and although he estimated the duration of larval life as three years, it seems certain that at least one additional year should be added to this value since Hardisty and Huggins (1970) showed that some ammocoetes from the third mode were still present after the time of metamorphosis. Manion (in Hardisty and Potter, 1971) reported that some landlocked Sea lamprey ammocoetes in a population comprising only a single year-class, had not metamorphosed five years after the first transformed animals appeared. Although Manion's results were derived from an artificial situation, they show that under exceptional conditions there is considerable variation in the age at metamorphosis of *P. marinus*. Further studies on *L. fluviatilis* populations may show to what degree this species diverges from the estimated four and a half years of larval life.

Electro fishing has revealed that following the metamorphosis of *fluviatilis* in early autumn, some transformed animals quickly emerge from the ammocoete beds and move to areas of gravel or weed, while others have been found in or near silt beds throughout the winter and

early spring. The appearance of downstream migrant *fluviatilis* in trawls made in the lower reaches of the River Severn during March and April, demonstrates that migration is occurring at that time, but does not indicate the peak of migration as there is no evidence on the intensity of migration elsewhen during the year. However, the fact that transformed animals have not been caught in the upper reaches after early April indicates that the exodus from the upper reaches is complete at that time. The spring downstream migration of landlocked Sea lampreys in the tributaries of the Great Lakes has been correlated with rising water levels (Applegate, 1950), and Gritsenko (1968) reported that spring flooding synchronised the downstream migration of *L. japonica* in Siberian rivers. If the downstream migration of *L. fluviatilis* in the River Severn is similarly correlated with flooding, then river discharge rates (Figure 8) suggest that some migration is to be expected throughout the winter, although the sudden, high floods of early spring may be responsible for peaks of migration.

Length-frequency distributions of juvenile *fluviatilis* adults caught by elver trawling in 1970 and 1971 show length ranges of 88 - 125 mm. and 84 - 133 mm. respectively. Although the distributions are based on small numbers, the 1970 distribution exhibits a marked bimodality with modes ranging from 88 - 112 mm. and 112 - 125 mm. The 1971 distribution is more complex, but still shows one well defined mode ranging from 84 - 106 mm., closely corresponding to the initial mode of the 1970 sample. Sexual dimorphism could be responsible for the bimodality, but this hypothesis could not be investigated as the animals were required alive for other studies. Variations in ammocoete

FIGURE 8



Histogram of average weekly discharge of River Teme at Tenbury Wells over the period 1968-1971

feeding conditions, or an extension of the larval period in a proportion of the population might also affect the length-distribution of downstream migrants.

In the spring, a high proportion of recently caught transformed *fluviatilis* were successfully transferred directly to, and maintained in full-strength sea water. Although transformed animals which had been caught in the autumn and maintained in the laboratory in fresh water over winter appeared less able to survive direct transfer, a high survival was recorded when lampreys from any source were subjected to short periods of acclimation in dilute sea water prior to transfer to full-strength sea water.

Bahr (1953) found that some young adult *fluviatilis* caught in the Elbe estuary were able to survive for at least five weeks in salinities of $33^{o}/_{oo}$. He concluded that at least a proportion of the *fluviatilis* population from this region had an unrestricted marine distribution, although other observations indicated that the "overall condition" of some young *fluviatilis* was affected by salinities of above $20^{o}/_{oo}$. Bahr regarded *fluviatilis* during its predatory phase as a brackish-water animal, and both he and Sterba (1962) have correlated the relatively large *fluviatilis* populations occurring in some of the rivers flowing into the Eastern Baltic Sea with the comparatively low salinity of that area.

Closed-system marine aquaria are always liable to accumulate toxic materials and bacteria, and are therefore unsuitable for long-term salinity experiments, but even under these conditions the fact that most of the recently transformed *fluviatilis* caught from the

Severn survived for several weeks following their direct transfer indicates that full-strength sea water does not limit their marine distribution.

The behaviour of some transformed *fluviatilis* undergoing acclimation indicated that there was a gradual emergence of previously burrowed animals during the period of increasing salinity. In cases where animals died following their transfer to full-strength sea water, death appeared to be linked to a condition involving considerable abdominal swelling, apparently caused by accumulation of water in the alimentary canal. Swallowing of water frequently occurs during the osmoregulation of teleosts which have been transferred from fresh to sea water, (Potts and Parry, 1964), and the abdominal swelling of some transferred lampreys may have resulted from an as yet unexplained accumulation of swallowed sea water.

Under laboratory conditions, transformed *fluviatilis* which had survived transfer from fresh to sea water did not feed on either live or dead fish. However, many of the animals did show behavioural patterns which could be related to the feeding behaviour of landlocked Sea lampreys described by Groot (1958) and Lennon (1954). Transformed River lampreys were induced to attach to dead fish, but the attachments only led to superficial damage. Lacerated, dead fish evoked the strongest behavioural responses, and recordings of the olfactory bulb activity (see Electro physiological studies) of upstream migrant *fluviatilis* showed that much bulbar activity is evoked when the olfactory epithelium is stimulated with fluid filtered from a suspension of mascerated fish flesh. The high proportion of lamprey brain devoted to olfactory function may indicate that lamprey behaviour is dominated

by olfactory information, and a small closed volume of water in an aquarium may soon become so saturated with odours that directional olfactory information is not possible. This arrangement might lead lampreys to exhibit some of their feeding behaviour and responses, whilst eliminating their ability to locate food. Groot (1958), on the basis of the ethological definitions of Tinbergen (1951) and Baerends *et al* (1955), has suggested that oblique swimming and re-location after attachment are forms of appetitive lamprey behaviour, while attachment and wounding are consummatory acts. The aquarium conditions during the preliminary feeding experiments with juvenile *fluviatilis* may have permitted appetitive behaviour patterns, but may have been unsuitable for the stimulation of consummatory acts. The complete absence of information on the marine life of *L. fluviatilis* from the rivers of the Bristol Channel area, and the difficulties experienced in laboratory studies of their marine feeding behaviour, leaves unobserved this important period in the life cycle.

After the capture of downstream migrants in the upper reaches of the estuary, River lampreys in their natural environment are not again available for study until they reappear on their upstream migration when they can be caught in large numbers. Hardisty (pers. comm.) has estimated the numbers of Severn upstream migrants in some years at 100,000. Returns of tagged downstream migrant salmon smolts show low returns (Harden-Jones, 1968) seldom greater than 10%. A salmon smolt tagging experiment in the rivers of the Bristol Channel region showed a return of only 0.3% (unpublished data, Min. Ag. Fish & Food). The reasons for low salmon returns are not known, but high mortality due to predation has been postulated (Harden-Jones, 1968).

In view of Hardisty's estimate, the numbers of downstream migrant *fluviatilis* must be very great if similarly low returns are characteristic of River lampreys.

The simple trapping method used at Tewkesbury weir to catch upstream migrating *fluviatilis* is based on observations that under most conditions lampreys have great difficulty in passing the weir, and can only surmount it easily during relatively high tides when the height of the waterfall is reduced. Other anadromous fish such as Salmon pass the weir by jumping over it. Information on the migrations of lamprey populations based on catches made at river barriers may be erroneous because of downstream accumulation of animals during low water conditions (Lanzing, 1959).

Another important factor in the successful capture of upstream migrants at Tewkesbury, stems from the observation that the lamprey migration in the lower reaches of the river is almost entirely nocturnal (Enequist, 1937; Tesch, 1967; Hardisty and Potter, 1971). Ryapolova (1964) has recorded that a reduction in the catches of migrating *fluviatilis* in Latvian rivers occurs on bright, moonlight nights, and in fact traps may not even be set at Tewkesbury under such conditions because the high light intensity is known to drastically reduce catches even though water levels may permit movement over the weir.

Because catches of lampreys at barriers may not accurately represent the movements of River lampreys through the estuary and lower reaches of a river, attempts were made to obtain information from other sources. The accidental capture of lampreys in the water intake

filters of power stations on the estuary provided some data, and in future work this source will be used to correlate the movements of lampreys with river and estuarine conditions.

Abakumov (1954) provided some information on the movements of upstream migrating *fluviatilis*, and concluded that some of the important factors involved were water levels and clarity, current velocity, relative sea and river temperatures, cloud cover and lunar phase. Bright moonlight reduced night-time catches, whereas heavy rain sometimes increased water turbidity to such a degree that day-time movement occurred. Increased downstream flow led to increased catches, as did high water levels which were thought to assist lampreys in overcoming "estuarine shallows".

Abakumov (1956) postulated that the diphasic (autumn and spring) River lamprey migration from the Gulf of Bothnia was dependent on climatic factors. The animals were found to gather around river mouths in the autumn, with a proportion of the population migrating upstream in the autumn, with others over-wintering in the estuary to migrate in the spring. The autumn movement of River lampreys from the Bristol Channel into the Severn may be correlated with both high tides and increased downstream flow during October and November. A further correlation with downstream flow is indicated by the occasional appearance of early autumn migrants passing through the estuary during periods of sudden summer flooding. Alabaster (1970) showed that the upstream migration of anadromous salmonids is directly related to river flow, and further postulated that salmon move upstream in response to a) short term changes in flow (freshets) irrespective of the previous flow rate, and b) accompanying changes in water quality associated

with freshets. Creutzberg (1958) has shown that the inshore and estuarine movements of elvers result from their behavioural responses to tidal flow and salinity conditions. In a later study he discovered that natural "inland" water was attractive to migrating elvers (Creutzberg, 1961).

Estuarine conditions, resulting from high turbidity and continual changes of salinity, flow rate and direction, provide few undeviating clues to anadromous fish moving through the area towards the river. However, a strong flow of fresh water moving into such a water mass almost certainly would provide directional information, and the apparent correlation between the time of autumn flooding and the time of the autumn estuarine migration of River lampreys may indicate that this animal responds to this information.

Within a river system, migrating lampreys are seldom observed until they appear on the spawning grounds in the spring. Migration in the middle and upper reaches of a river system is nocturnal (Wikgren, 1953; Abakumov, 1957) and Sterba (1962) assumes that the animals spend the day hiding under stones and overlying banks. It is possible that some autumn migrants reach the spawning grounds well in advance of the spring spawning period, and Sterba's (1962) assumption that these animals hide nearby was borne out by the capture of immature animals in early spring hiding under stones at Ashford Carbonnel on the River Teme. These animals were caught below a weir which was thought to be almost impassable for migrating River lampreys. Even salmon find difficulty in passing this weir, and much rod and line salmon fishing takes place immediately downstream of the weir (Ayton, Severn River Authority, pers. comm.).

In both the Teme and the Severn, River lampreys have been found spawning on gravel beds downstream from large and presumably impassable weirs. "Traditional" lamprey spawning grounds appear therefore, to be related to the construction of high weirs, and because migratory lampreys are less able to pass weirs than other anadromous fish, it could be argued that the extensive construction of weirs and other river obstructions in the 19th century (Ashworth, 1868) may be a factor in the decline of European lamprey stocks. This factor was apparently overlooked by Sterba (1962) who in the main attributed the decline to the pollution of rivers with special emphasis on sewage pollution of ammocoete substrates.

In the years investigated (1969 - 1971), the spawning period of *fluviatilis* in the Teme was well defined, in that it occurred in all three years during a period of low water flow associated with increasing water temperatures (Figure 6). River conditions are dependent on climatic conditions, and the period of relatively dry, sunny weather which apparently occurs in most years during late March and April results not only in low water levels, but also in increased water temperatures.

It has been shown by Damas (1950), Bahr (1953) and Hagelin (1959) that certain conditions are essential if lampreys are to be encouraged to spawn in aquaria. For example, it is necessary to provide a gravel substrate and flowing water. Sterba (1953) showed that *L. planeri* could be considered as "stenophotic" during spawning because in his experiments, spawning animals sought out and followed a patch of bright sunlight which he was able to direct on to a stream. Observations of *fluviatilis* spawning in the Teme indicated that water

temperature was important in relation to phases of spawning behaviour and although no release of milt or eggs was seen at temperatures below 10.5°C, lampreys were observed nest-building at temperatures above 8.0°C, while on one occasion they were present but inactive on the redds at 6.0°C. Extreme diurnal temperature variation, or sudden climatic changes may have been responsible for some of the widely differing spawning temperatures which have been recorded for *fluviatilis*, e.g. Lauterbourn (1926) 8.7°C, Gaygalas and Matskevichyus (1968) 15.4 - 17.2°C.

The spawning of *fluviatilis* on the Tywi was difficult to observe because the river has no weirs to obstruct the upstream migration of lampreys, and throughout its length it runs shallowly over gravel. Comparison of river authority annual water temperatures for the Tywi and Teme has shown that the Tywi is slower to warm up during the spring, and in all three years, the few spawning *fluviatilis* taken from the Tywi were caught 1 - 3 weeks after the peak of spawning in the Teme. Captured Tywi *fluviatilis* were smaller than most of those taken from the Teme, (see Figure 7), and it is suspected that the upstream migration in the Tywi is mainly a spring movement because the animals had only been seen by local estuarine fishermen in the spring. There was no large-scale spring migration of *fluviatilis* in the Severn during the years of this study, but some adult River lampreys were caught during elver trawling in the spring. These animals were held in the laboratory where they survived longer and matured later than similarly held autumn migrants. One of the spring-caught animals was maintained for 3 weeks in full-strength sea water, and it is possible that some spring migrants are not fully committed to a spawning migration. The small size of some Severn spring migrants may indicate that they have spent a shorter marine phase than most autumn migrants.

In the Tywi, spawning *fluviatilis* were always accompanied by *planeri* in the redds. On several occasions *planeri* were also found in the redds of Teme *fluviatilis*. Observations of this communal spawning in the Tywi showed that accidental hybridisation may have occurred through the simultaneous shedding eggs and milt by both species. *planeri/fluviatilis* hybridisation has been easily achieved by artificial fertilization, and the resulting ammocoetes have been reared for some years, although not to metamorphosis. It is interesting to speculate on the length of larval life, osmotic ability and adult feeding of such hybrids, but only until the hybrids have been successfully reared will such questions be answered.

Although river conditions allow *planeri* to successfully spawn in or near *fluviatilis* redds in the Tywi, the *fluviatilis* redds on the Teme are unsuitable for *planeri* spawning because of the deeper and faster water, and the nature of the gravel. The presence of *planeri* in Teme *fluviatilis* redds therefore raises the question of how and why *planeri* are attracted to the area. In fact the group spawning of *fluviatilis* has yet to be explained since the assembly of the animals at one time in one region of the river system is unlikely to be random, and therefore requires some mechanism.

The eyes of lampreys, although not rudimentary as was earlier thought by Kohl (1892), are certainly primitive (for review see Kleerekoper, 1972), and in aquaria, the animals frequently "bump" into obstacles. Groot (1958) noted that lampreys when attacking fish frequently miss their prey, and concluded that "visual stimuli cannot direct the searching behaviour of the Sea lamprey.....". Olfaction may well be the important sense in the aggregation of spawning groups and

Morris (1957) working on *fluviatilis*, discovered "male glandular cells" which were only apparent in the gill epithelium of sexually mature males. Morris postulated that these cells secreted "some substance of sexual significance, presumably connected with spawning". Fontaine (1938) recorded that French lamprey fishermen used sexually mature male Sea lampreys as lures in traps in order to capture female Sea lampreys. Sterba (1953) stated that spawning animals appeared to be "chemo-tactically" attracted to other spawning groups.

The above evidence indicates that assembly of spawning groups of lampreys probably occurs as a result of olfactory attractants exuded by sexually mature animals, and it is interesting that the communal spawning of the closely related *planeri/fluviatilis* pairs may occur as a result of *planeri* responding to sexual attractant released by spawning *fluviatilis*.

III

ELECTROPHYSIOLOGICAL STUDIES

III ELECTROPHYSIOLOGICAL STUDIES

A. INTRODUCTION

i) Brain Activity in Vertebrates:

Following the development of the string galvonometer and the cathode ray oscilloscope in the first quarter of this century, electrophysiology developed rapidly. Early studies of the human electroencephalogram, obtained by amplifying electrical brain activity recorded from scalp electrodes, were made by Pravdicz-Neminski (1925) and Berger (1929, 1930). Clinical applications of the method were soon devised, and led to the discovery of epileptic rhythms in brain activity (Gibbs *et al*, 1935) and to the use of electroencephalographic techniques to localise brain tumours (Walter, 1936). The rapid progress of electronic technology has led to sophisticated developments of the electroencephalograph with the result that it now commands clinical importance in many medico-physchological investigations. The human electroencephalogram exhibits four basic types of rhythmic waveform activity which have been termed alpha (8 - 13 Hz), beta (more than 13 Hz), delta (1 - 4 Hz), and theta (4 - 7 Hz) waves, (Walter, 1953).

Rhythmic brain activity has also been recorded from a number of lower vertebrates by the topical application of macroelectrodes onto the surface of specific brain regions. Thus, Veselnikin (1963) recorded some rhythmic electrical activity from the brain of the

River lamprey and Gilbert *et al* (1964) recorded activity patterns from the brains of sharks. Shurrager (1936) recorded activity from the isolated forebrain of the catfish, a study which had been preceded by the work of Adrian and Buytendijk (1931) on the isolated brainstem of the goldfish. Schadé and Weiler (1959) also recorded activity from the goldfish brain and showed that anaesthesia lowered both the frequency and amplitude of spontaneous potentials. Enger (1957) described the electroencephalogram of the cod during an attempt to discover the effects on the brain of high frequency mechanical vibrations similar to those emitted by echo sounders.

Studies on the brain activity of amphibia began with the work of Gerard and Young (1937) and Libet and Gerard (1939), who considered that the olfactory lobes of the frog brain were the electrically dominant structures, a view which was endorsed by Segura and de Juan (1966) who investigated seasonal variations in the spontaneous brain activity of toads. Goodman and Weinberger (1969) studied the brain activity of the mud puppy, and Peters and Vonderahe (1954)investigated electroencephalographic effects of pharmacologically-induced muscular seizures in salamanders.

An early electroencephalographic study of the cortical region of the reptile brain was made by Bremer *et al* (1939) and Parsons and Huggins (1965) carried out a more detailed study of the spontaneous brain activity of the caiman.

A summary of some of the above studies (Table 4) shows a conformity of activity in the same brain regions of different animals, telencephalic activity being very similar in all the studies.

TABLE 4

ANIMAL	SHARK	GOLDFISH	GOLDFISH	COD	FROG	TOAD	SALAMANDER	SALAMANDER	CAIMAN
AUTHOR (S)	Gilbert *et al* (1964)	Adrian and Buytendijk (1931)	Schadé and Weiler (1959)	Enger (1957)	Gerard and Young (1936)	Segura and de Juan (1966)	Goodman and Weinberger (1969)	Peters and Vonderake (1956)	Parsons and Huggins (1965)
Telencephalon (mainly olfactory lobes)	4 – 9 Hz 30 – 60 μV	–	4 – 8 Hz 40 – 70 μV	4 – 6 Hz –	3 – 8 Hz 20 – 80 μV	7 – 11 Hz 150 – 500 μV	4 – 30 Hz 50 – 150 μV and 4 – 6 Hz 20 – 50 μV	5 – 8 Hz and 12 – 14 Hz	7 – 12 Hz 20 – 40 μV and 18 – 24 Hz 10 – 20 μV
Mesencephalon (Optic lobes)	In light: less than 40 μV In dark: 5 – 11 Hz 80 – 170 μV	–	In light: 18 – 24 Hz at "low amplitudes" In dark: 7 – 14 Hz 40 – 180 μV	In light: 14 – 32 Hz 5 – 15 μV In dark: 8 – 13 Hz 20 – 160 μV	45 Hz less than 50 μV	Low frequencies (less than 1 Hz) "high amplitudes"	Feeble baseline changes 1 – 2 Hz 5 – 15 μV	–	–
Myelencephalon (Medulla Oblongata)	Potentials related to respiration 150 – 200 μV	Isolated brainstem showed slow rhythmic potential changes approximately coincident with opercular beat.	Low amplitude potentials related to respiration	14 – 32 Hz and low frequency potentials recorded synchronously with respiratory movements	Spike-like rhythmic activity 2 Hz 50 – 60 μV	–	–	–	–

Nikitsvenka (1964) correlated the phylogenetic origins and mode of life of fish with their brain structure and Teichmann (1954) has classified fish on the dominance of either olfaction or vision in their behaviour. The electrical dominance of one brain structure over another may indicate the relative importance of certain senses or abilities in the behaviour and mode of life of an animal and thus Segura and de Juan's (1966) recordings of activity from the toad brain (Table 4) may indicate that the olfactory sense dominates the behaviour of the toad. Hara *et al* (1965) used Teichmann's (1954) classification in conjunction with their recordings of the brain activity of salmon, to postulate that this fish successively progresses through three different stages in its life cycle. Young, freshwater salmon exhibited high activity in both their olfactory (8 - 10 Hz, 35 - 65 μV) and optic (9 - 10 Hz, 25 - 50 μV) lobes. The olfactory lobes of upstream migrating ripe salmon exhibited more activity than their optic lobes, which in some cases were "electrically silent". Young salmon were therefore assumed to possess equally well-developed visual and olfactory senses, while upstream migrants had a better olfactory than optic sense. The marine stage of the salmon was known to involve predatory feeding and sun-compass migration (Hasler, 1966), and therefore the visual sense was assumed, during this phase, to be the most important.

ii) Olfactory Lobe Activity and Homing in Salmon:

Hara *et al* (1965) infused various natural water samples into the olfactory sacs of adult spawning salmon, and recorded olfactory lobe responses. River water, from sources other than the home tributary, evoked little or no change in olfactory lobe activity. Home river water, i.e. water taken from the region of the river in which the salmon was spawning, evoked vigorous bulbar responses, from which it was postulated that olfaction was important in orientation during the homing migration. The necessary olfactory discrimination was thought to occur at either the epithelial or bulbar level.

Although Hara *et al* demonstrated that home water evoked a distinct response in the olfactory bulbs of spawning salmon, the fish were collected from only one spawning site at which odourous substances, may, by accident, have been in high concentration. In a later study (Ueda *et al*, 1967), olfactory bulb responses of spawning salmon taken from three different spawning grounds were recorded following stimulation with home and other river waters. This study re-emphasised the specific home water response, and demonstrated that similar responses could not be evoked by water from the spawning sites of other salmon. Weaker but distinct responses were also evoked by i) water from upstream of the spawning site, ii) water from a by-passed tributary near to the spawning site and iii) waters traversed by the salmon during its anadromous migration.

Hara *et al* (1965) found that home water lost its stimulant action if it was diluted more than 10 times by tapwater. As water from the higher reaches of a river is greatly diluted in the river system, a theory of olfactory homing, based on the results of Hara *et al* (1965)

therefore required that homing salmon should sequentially respond to the waters at various "loci" along the freshwater migratory route. Oshima *et al* (1969 a) attempted to demonstrate that this occurred by testing salmon from three spawning sites with water taken from i) the spawning grounds, ii) the migratory routes, iii) by-passed tributaries and iv) non-related river systems. In two of the groups of salmon, freshwater samples taken from the migratory routes evoked distinct bulb responses, all of which were less than the home water response, while in the third group, "traversed water" responses were of the same magnitude as those evoked by spawning site water.

A further study by Oshima *et al* (1969 b) showed that in one case, a non-home water was as stimulating as home water to spawning salmon from two different spawning sites. They also discovered that even after being kept in sea water for two weeks, young salmon readily responded to home water which evoked a different response to that evoked by other natural waters. Non-home water was found to be highly stimulating to young salmon if it had contained salmon of the same species and in fact, Oshima *et al* (1969 b) thought their experiments suggested that the olfactory components in home water of the young salmon were largely derived from the fish themselves. In this context, the observations of two experienced Scottish salmon fishermen has led them to postulate a theory that decomposing spent salmon impart a characteristic smell to a river which "attracts" subsequent returning salmon (Linklater, 1969).

The review by Hara (1970) fully explores the electro-physiological evidence for olfactory homing in salmon and concludes that

water from the home spawning site of a salmon evokes a characteristic olfactory bulb response which is of greater magnitude than the responses evoked by water taken from other spawning sites.

What then are the olfactory stimulants in home water to which salmon must respond in order to return to their home spawning site? That odours can affect the behaviour of fish was first demonstrated by von Frisch (1941) who showed that extremly dilute "emanations" from injured fish skin can cause alarm and dispersion in fish schools. As previously stated, Oshima *et al* (1969,b) showed that the stimulant action of water was increased if salmon were kept in it and Walker and Hasler (1949) trained bluntnose minnows to detect and discriminate among the odours imparted to water by aquatic plants. Further conditioning experiments (Hasler and Wisby, 1951) showed that these fish were capable of distinguishing between water samples taken from two streams draining from different edaphic regions. Fish which had been conditioned to a particular natural water were then presented with fractions of that water, and the chemicals utilized by the fish for odour recognition were thought to be aromatic compounds which were volatile at 25^{o}C. On the basis of these results, Hasler and Wisby (1951) concluded that natural waters contain volatile organic compounds which can be olfactorily detected and recognised by fish, even after periods of non-exposure.

III) Theories of Olfaction:

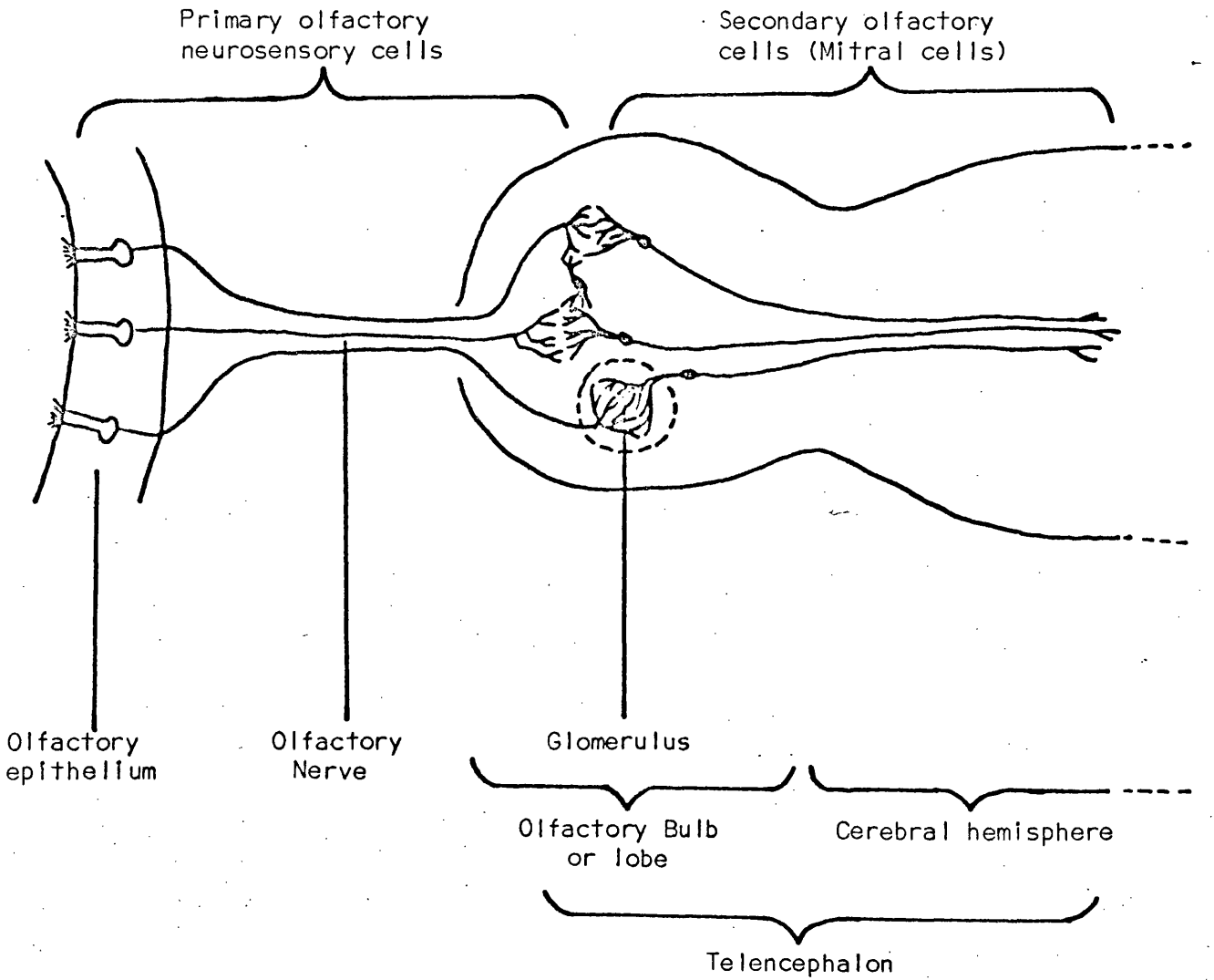
The basic mechanism of olfactory stimulation by chemical substances is still not fully understood, although several theories have been postulated. The absorption or radiation of energy by odourous molecules has been thought to stimulate olfactory receptor cells, and the infra-red theory of Beck and Miles (1947) assumed that the odourous qualities of different molecular structures depends on their absorption of heat energy. However, Ottoson (1956) showed that when a thin membrane, capable of transmitting heat and light is placed over olfactory receptor cells, they are not activated by odourous molecules. Perhaps the present, most widely held theory of olfaction is the stereochemical theory first suggested by Moncrieff (1949) and elaborated by Amoore (1962,a,b) and Amoore *et al*, (1964), which suggested that part of the surface of olfactory receptor cells is composed of "pits" into which can fit only certain molecules. Seven basic odour qualities are thought to exist, five of which depend on molecular shape, and two on electrical characteristics. At present, the stereochemical theory appears to be consistent with evidence relating to olfaction, and although there is disagreement on the nature and number of basic odours, Johnston and Sandoval (1960) have correctly predicted the odour qualities of newly synthesised compounds on the basis of their molecular shape.

iv) The Structure of The Olfactory System:

Le Gros Clarke (1956) suggested that odour-perceptor sites are located on the many cilia of olfactory receptor cells, and de Lorenzo (1963) showed that in the olfactory chamber, these are the only parts of the sensory cells which are exposed to the environmental medium. The relationship of receptor cells within the epithelium to the olfactory nerve fibres, has been the subject of much discussion (see Kleerekoper, 1969). However, it has been established that the olfactory receptor cell is the primary bipolar olfactory neuron, with peripherally directed dendrites (cilia) and extremely thin axons which pass from the epithelium to the olfactory brain without interconnecting neurons.

These axons enter an extension of the telencephalon known as the olfactory bulb. In the superficial zone of this bulb, the axons interlace in a complex pattern with the branched terminations of secondary olfactory neurons (mitral cells) to form spherical complexes known as glomeruli, in which synaptic transmission occurs. Scattered among, and connecting the glomeruli, are small stellate neurons known as spindle cells. Axons of the mitral cells form the secondary olfactory pathways into the cerebral hemispheres. The general vertebrate scheme of olfactory neural organisation, based on information from the review of the comparative anatomy of olfactory centres and tracts by Nieuwenhuys (1967) is shown in Figure 9.

FIGURE 9 Diagrammatic representation of vertebrate olfactory system



v) The Electrophysiology of the Olfactory System:

The fundamental structure of the olfactory system does not vary markedly throughout the vertebrates, and displays similar electrophysiological characteristics in all groups. Thus, activation of receptor sites on the cilia-like dendrites of the primary cell results in a relatively slow and long-lasting generator potential in the cell body (Ottoson, 1956) and spike discharges in the long axon (Gesteland *et al*, 1963).

The olfactory bulb, in the absence of olfactory stimulation, exhibits spontaneous irregular activity of relatively high frequency. Olfactory stimulation evokes regular, lower frequency, higher amplitude waves superimposed on slow potentials (Ottoson, 1959). The slow potential, probably arises in the glomeruli, and unit recordings from the bulb during stimulation show that cellular discharges occur in synchrony with the waves (Adrian, 1950).

vi) Olfactory Discrimination:

Investigations of the mechanisms of olfactory discrimination have indicated that this may occur at both the epithelial and bulbar level. Adrian (1950) suggested that receptor cells were spatially differentiated on the epithelial surface within the nasal chamber, and Gesteland *et al*, (1963) demonstrated that the olfactory receptors of the frog were odour-selective. In this context, Matthews and Tucker (1966) were able to show that in the tortoise, single units of the olfactory epithelium exhibited highly individual sensitivity patterns.

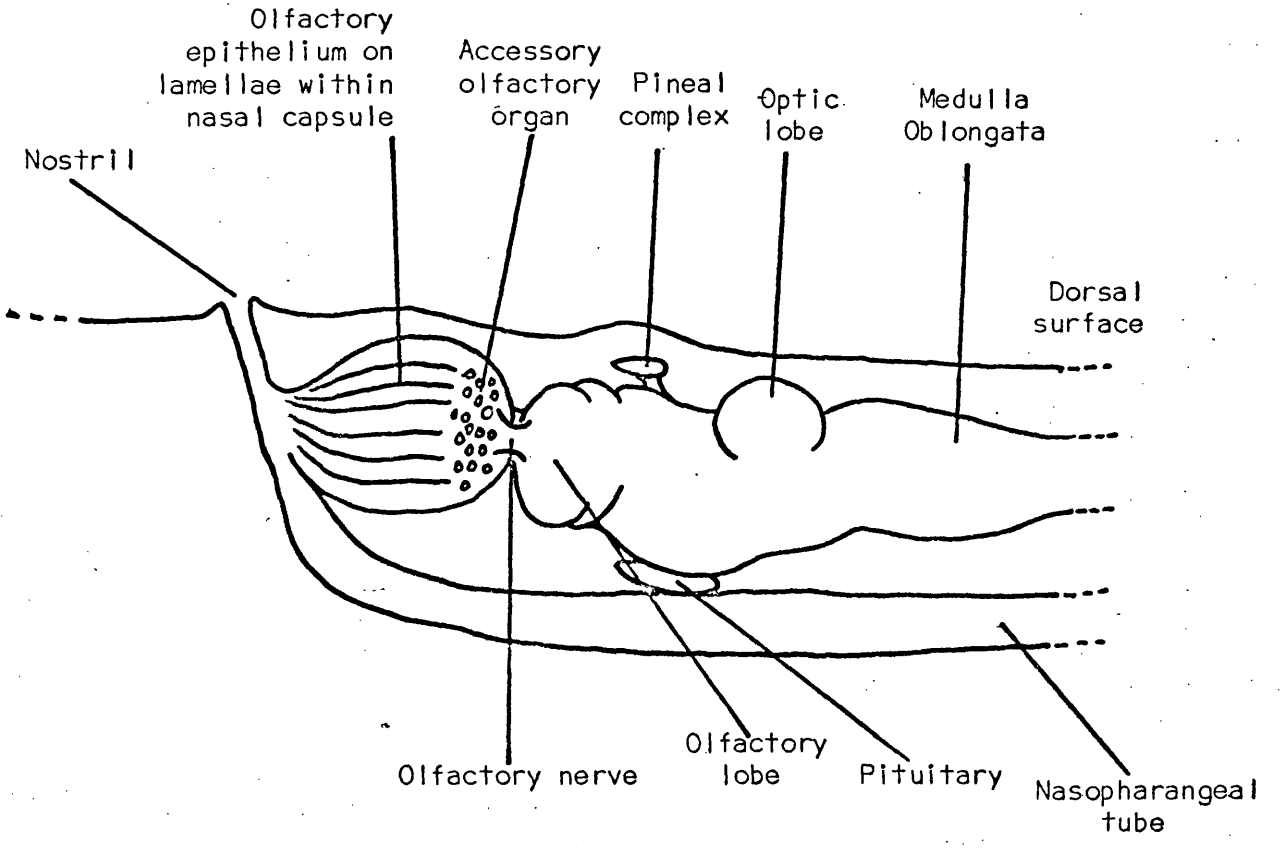
The mitral cells of the olfactory bulb and their areas of synaptic connection (glomeruli) are also thought to be involved in olfactory discrimination. For example, Leveteau and MacLeod (1966) recorded slow, monophasic potentials from individual glomeruli of the rabbit olfactory bulb and reported a degree of discrimination in the responses of these structures, and Philips and Michels (1964) showed that the activity of the mitral cells within the olfactory bulb of the opposum was dependent on the type of stimulant and on the position of the cell within the bulb. In the rabbit, an estimated 50 million epithelial receptors relay olfactory information through approximately 2,000 glomeruli to 5,000 mitral cells (Allison and Warwick, 1949). This ratio of receptors to mitral cells is similar to the ratio between receptors and secondary myelinated olfactory fibres in the tract of the burbot (Døving and Gemne, 1963) and the integration of olfactory information may thus be based on this pattern of convergence.

vii) The Olfactory System of the Lamprey:

No studies of the ratio of primary to secondary olfactory neurons have been attempted on the olfactory system of lampreys, although the monorhinic olfactory system of cyclostomes has attracted much attention (see Kleerekoper, 1969). A single, median-dorsal nostril opens via a short nasal tube into both the olfactory capsule and the nasopharyngeal pouch (Figure 10). Lampreys have only one nostril and one olfactory sac, but in the adult, the lumen of the capsule is incompletely divided by a median septum. Lamellae which project into each half of the capsule support the olfactory epithelium, and in both the ammocoete and the adult, two olfactory nerves leave the cartilaginous wall through separate openings and almost immediately enter the paired olfactory lobes.

The monorhinic condition of cyclostomes has been the cause of speculation about whether this olfactory system is a "phylogenetic link" between the single olfactory groove of *Branchiostoma (Amphioxus)* and the amphirhinic condition of gnathostomatous vertebrates. Most embryological studies of lampreys have shown a single precursor of the olfactory organ (Scott, 1887; von Lubosch, 1901; Gerard, 1954) and the observation of paired structures by Calberla (1877) has never been confirmed. The precursor initially takes the form of a ciliated groove, and von Kolliker (1843) postulated that this might be the homologue of the ciliated olfactory groove of *Branchiostoma*. von Kupfer (1894) reported a further development of additional lateral precursors, and considered that the monorhinic cyclostome condition was intermediate between the *Branchiostoma* situation and amphirhinic gnathostomes.

FIGURE 10 Diagrammatic median sagittal view of lamprey brain and olfactory system



However, Peter (1925) was not able to confirm the development of the additional lateral precursors.

Dohrn (1875) was the first to suggest that monorhiny was a secondary development and considered that the paired olfactory nerves indicated an earlier amphirhinic condition which had been lost with the development of the parasitic habit. The cyclostome monorhinic condition is now mainly regarded as a secondary development (Scott, 1887; Cords, 1929; Kleerekoper and van Erkel, 1960).

As previously indicated, the olfactory epithelium of the ammocoete is ciliated and single-layered. During metamorphosis, this epithelium becomes multilayered, and numerous longitudinal folds develop and project into the lumen of the capsule (Kaensch, 1890). Sensory epithelium in the adult is limited to the lateral walls of the lamellae (Ballowitz, 1904) and is composed of sensory cells, supporting cells and basal cells (Thornhill, 1967).

The posterior region of the olfactory sac is highly vascularised and contains much connective tissue. Within blood sinuses are gland-like aggregates of cells in vesicles (Figure 10), forming a structure known as the accessory olfactory organ, (de Beer, 1924; Hagelin and Johnels, 1955). Only a few such vesicles are found throughout larval life, but at metamorphosis there is a rapid proliferation in their numbers (von Lubosch, 1905; Imamura, 1928). The function of the accessory olfactory organ of cyclostomes is not known, although its glandular appearance had led to comparisons with the Bowman's gland of mammals (de Beer, 1923; 1924; Leach, 1951), and its nervous supply suggests a sensory function (von Lubosch, 1905; Hagelin and Johnels, 1955).

viii) Olfaction and Behaviour in Lampreys;

The development, in the adult lamprey, of a massive and highly specialised olfactory apparatus indicates that olfaction is a major sensory mechanism during the post-metamorphic stage of the life cycle. Using an ingenious monitoring technique involving the use of a compartmented tank, Kleerekoper and his co-workers have analysed the locomotory behaviour of parasitic land-locked Sea lampreys when exposed to the odour of prey fish (for review of work see Kleerekoper, 1969). When adult lampreys were presented, in the light, with water in which trout had been kept, their marked circadian rhythm of activity (Kleerekoper *et al*, 1961) was much altered through greatly increased locomotor activity during this normally inactive period (Kleerekoper and Mogensen, 1963). Juvenile lampreys, normally relatively inactive, which had presumably not encountered the smell of fish prior to the experiment, exhibited prolonged activity when presented with trout water, thus indicating that the response is innate (Kleerekoper and Mogensen, 1963). Anosmic lampreys exhibited no locomotor response to the smell of the fish.

An analysis of the chemical composition of trout scent in water (Kleerekoper and Mogensen, 1959; Kleerekoper, 1969) revealed that many chemicals are present, including ammonia, aminoacids, amines and other related compounds. In a series of experiments in which many mixtures and isolated chemicals were presented to lampreys, only two of the component chemicals, initially code-named amines C and F evoked locomotor responses similar to those evoked by trout water. Amine F, later identified as isoleucine methyl ester (Kleerekoper, 1972),

was more effective than amine C, and analysis of the body odours of 32 species of fish has shown that isoleucine methyl ester is present in all cases in varying degrees (Kleerekoper and Mogensen, 1963), and is effective as an attractant to several other fish species (Kleerekoper, 1963; 1967). The apparently widespread occurrence of isoleucine methyl ester and its effects on the loco - motor activity of several species of fish suggests that the chemical is a general attractant in the predator/prey behaviour of fish (Kleerekoper and Mogensen, 1963).

ix) Olfactory Thresholds:

The threshold concentrations of isolated chemicals which evoked behavioural effects in lampreys were found to be subject to seasonal variations, and Kleerekoper and Mogensen (1963) noted that the reduced seasonal responses coincided with a seasonal decline in the frequency of feeding attacks made by sexually maturing lampreys which were undergoing considerable physiological changes. In this context, Le Magnèn (1948) and Schneider *et al* (1958) have shown that olfactory thresholds in women are directly related to hormonal changes during the menstrual cycle and Hara (1967) showed that administration of sex hormones to goldfish directly affected olfactory lobe activity in that responses to stimulation with sodium chloride were of greater magnitude following the administration of estradiol.

Olfactory thresholds also appear to be linked to food intake, diet and blood sugar levels (Goetzl and Stone, 1947; Goetzl *et al*, 1950), and olfaction has been shown to play an important role in the regulation of food intake (Le Magnèn, 1956). The functional afferent and efferent paths between the olfactory region of the brain and other brain regions have only recently been investigated. Kandel (1964) has shown that stimulation of the olfactory tract of the goldfish can evoke responses in the neuro secretory cells of the hypothalamus and Døving and Gemne (1963) identified efferent fibres in the olfactory tract of the burbot. Central regulation of olfactory thresholds is therefore possible, and the study on anurans by Takagi (1962) showed that olfactory acuity is affected by the activity of other areas of the central nervous system.

Olfactory thresholds also depend on the method of presentation of the olfactory stimulants, and Neuhaus (1956) showed that threshold values in the dog depended on whether the stimulant was administered in isolation or in mixtures. As all naturally occuring odours are complex chemical mixtures, this factor is of some importance in dealing with the behavioural aspects of olfaction. Although olfactory thresholds have been shown to be dependent on several factors, attempts have been made to determine in fish the lowest odour perception levels of both complex scents (Bull, 1930; Newarth, 1949; Hasler and Wisby, 1950; 1959), and isolated compounds (Marcstrom, 1959; Teichmann, 1959). The work has been reviewed by Kleerekoper (1969), but the studies of olfactory thresholds in the eel by Teichmann (1959) deserve special attention as they illustrate the sensitivity of the olfactory sense. Eels were trained to respond to various chemicals in water and the lowest concentrations at which responses could be evoked were measured. Teichmann found that the eel responded to concentrations of β-phenyl ethyl alcohol equivalent to the presence of only one molecule of the chemical in the nasal chamber.

Work of this type indicates the high level of olfactory sensitivity of some fish, and the behavioural and electrophysiological investigations of olfactory homing emphasises the importance of the olfactory sense in their behaviour. Kleerekoper (1969) showed that olfaction is of great importance in the feeding behaviour of predatory adult lampreys, although electrophysiological studies of the sensory mechanisms involved were not carried out.

Because the life history of the anadromous River lamprey shows

clear similarities to those of anadromous salmonids and includes a period of intense predatory feeding based mainly on olfactory information, electrophysiological investigations of olfactory brain activity in River lampreys have been carried out. Olfactory bulb responses to home and other natural waters were recorded, as were quantitative responses to concentrations of chemicals possibly involved in lamprey behaviour. The results will be discussed in relation to the behaviour and life history of the River lamprey, and will also be compared with results obtained by other workers from similar studies of other fish.

B. MATERIALS AND METHODS

i) Recording Technique:

The electrical activity of the lamprey brain was recorded through an electrode placed directly onto the brain surface. Activity was then conducted to a high sensitivity preamplifier (Devices AC7C) for filtration and amplification, and further amplified by a power amplifier (Devices DC5). The resulting electrical changes were displayed as a permanent trace by a heat pen recorder (Devices M-2) with an upper frequency limit of 100 Hz.

Figure 11 diagrammatically shows the recording system. Two amplification stages were employed because although it is sometimes possible to feed a signal directly into a power amplifier, a pre-amplifier is preferable to match the source impedance to that of the power amplifier. After preamplification, activity was conducted through two leads into:

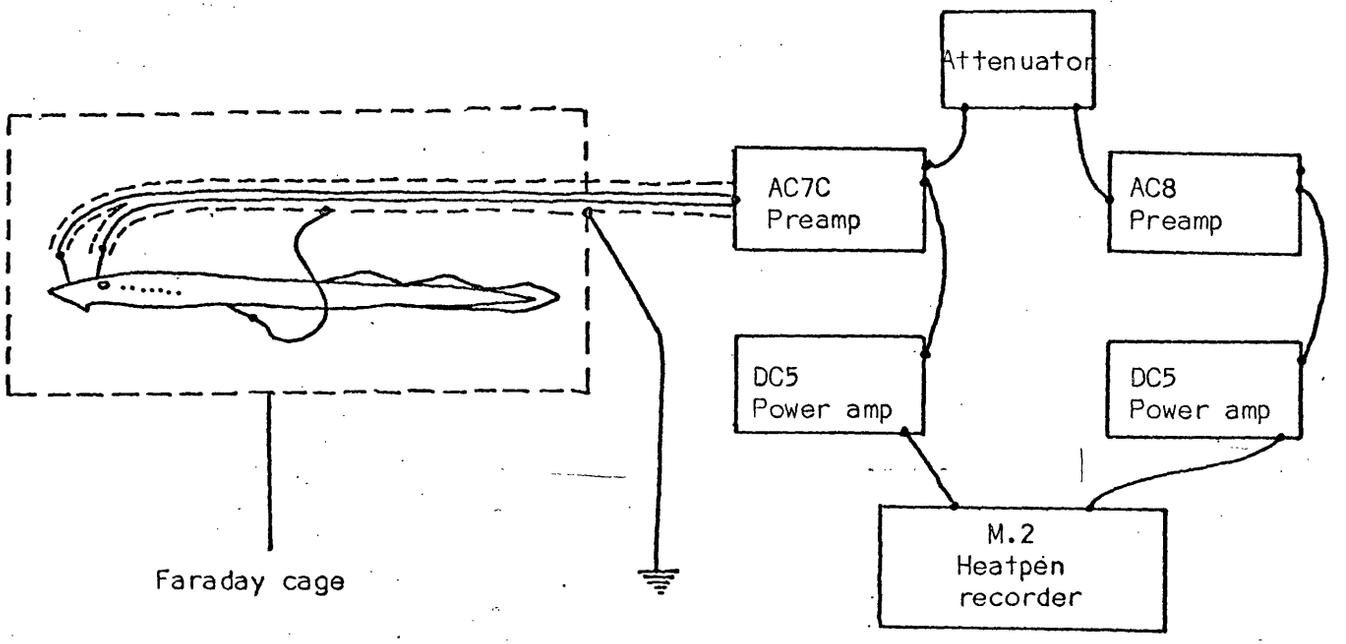
a) a power amplifier and thence into one channel of the pen recorder,

and

b) through a simple attenuator into another preamplifier (Devices AC8) which integrated activity before it was conducted to the second channel of the pen recorder.

Care was taken throughout the work to avoid electrical artefacts which could arise when high sensitivity apparatus is used in a busy laboratory. A large Faraday cage was constructed of wood and perforated

FIGURE 11 Diagrammatic representation of the recording system



zinc in which a stable work bench was erected. This wooden bench was independently supported, so avoiding much of the mechanical vibration normally present in ordinary laboratory benching. Mechanical vibration can be transformed into electrical interference through its action on the rigidity and position of the electrode.

The Faraday cage "screened" out induced voltages which otherwise enter the recording apparatus from adjacent electromagnetic fields. Most interfering noise has the basic frequency of the mains supply (50 Hz) although harmonics and other frequencies may occur. The perforated zinc acts as an aerial and interfering electromagnetic fields are conducted to earth. As the earth of the laboratory mains supply was unstable, a true earth consisting of a thick copper plate was sunk into a pit filled with coke. The coke was watered before experiments to ensure a good electrical contact between the copper and the surrounding earth. All earth connections for the recording apparatus and screening were made to a single point on this earth, and by arrangement of the positions and lengths of conducting cables, earth loops were avoided.

The Faraday cage was large enough to accommodate the work bench and the preparation, which outside the cage exhibited noise of greater amplitudes than the low level brain activity. The signal to noise ratio was acceptable during experiments carried out in the Faraday cage under the conditions described.

ii) The Electrode:

A silver/silver chloride electrode was used. Electrodes of this type have been widely used for recording brain activity (Enger, 1957; Schadé and Weiler, 1959; Gilbert *et al*, 1964; Hara, Ueda and Gorbman, 1965; Segura and de Juan, 1966). In principle, the electrode is a bridge between the brain and the conducting lead to the preamplifier. It consists of a body of highly conductive pure silver, intimately covered at the tip with silver chloride. This covering, when applied to the moist brain surface, forms an electrochemical bridge between the chloride ions of brain fluid and the metallic silver of the electrode. The Ag/AgCl electrode is non-polarisable in that it allows a free interchange of chloride ions whilst remaining qualitatively unchanged, and is toxic only in long-term experiments.

The electrode holder was a two-pin electrical plug (Plate 9). The electrodes were attached to the plug by two-ended solder tags, whose cylindrical construction allowed them to be firmly pushed onto the metal pins of the plug. The ends of each solder tag were bent over, and onto one end a 3 cm length of 0.1 mm diameter silver wire (Johnston-Matthey Metals Ltd., Grade 5 silver wire) was soldered. After the wire had been degreased and cleaned with diethyl ether, clear nail varnish (Cutex Spillproof polish, Cheeseborough Ponds) was applied to the shank of the electrode, insulating it except for a 0.1 mm exposed tip. Three coats of varnish were applied to the electrode shank, each being allowed to dry before the next was applied. By illuminating the electrode during the varnishing process, the dark

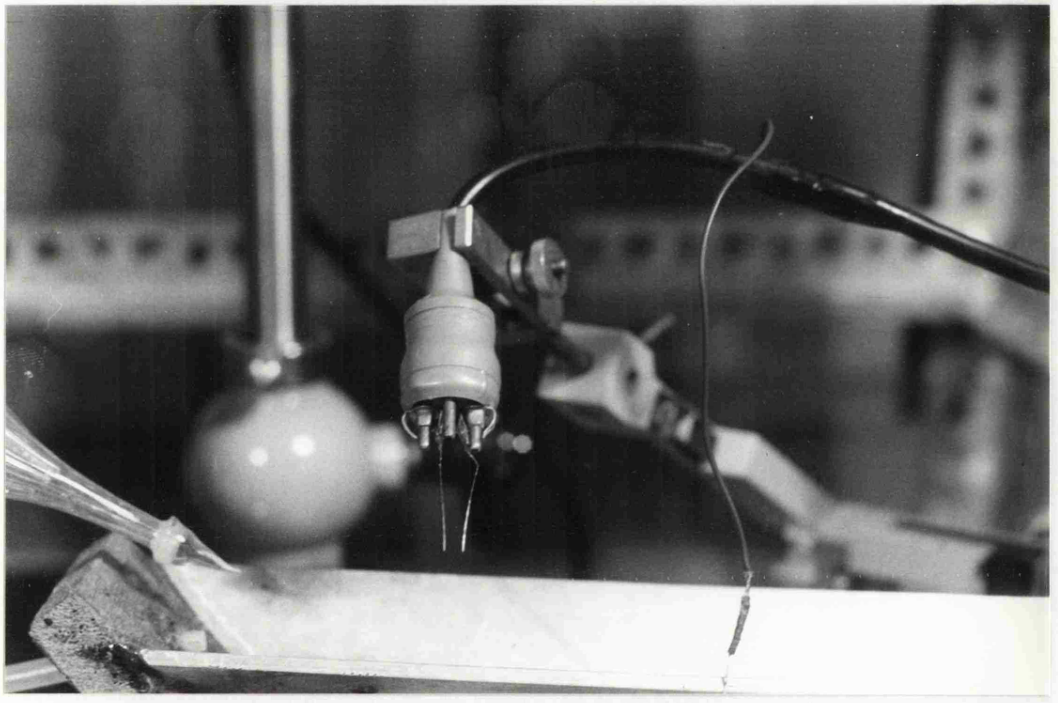


PLATE 9

Showing the electrode holder with attached electrodes. To the left of the holder is the stimulator and below is the trough support for the preparation.

metallic tip could be clearly seen in contrast to the varnish. The coating was achieved by pulling the electrode through a small brush charged with varnish.

Silver chloride was deposited on the exposed tip by making the electrode the anode in 0.1 N HCl electrolyte, with another silver wire as the cathode in a circuit completed by a 2V accumulator. Current was passed through the circuit for 30 sec. The electrode was then switched to become the cathode for another 30 sec. and the coating was completed by again reversing polarity making the electrode the anode for a final 30 sec. (see Silver in Donaldson, 1958). Electrodes were quickly and easily made, and because they were kept moist and used immediately after construction, no precautions against photochemical changes were necessary. Mechanical damage of the silver chloride layer was avoided.

iii) The Recording Apparatus:

The AC7C and AC8 preamplifiers are identical in that both are high input impedance, high sensitivity a.c. coupled amplifiers. However the AC8 has an additional circuit integrating high frequency signals for observation as a d.c. level on the pen recorder. Both preamplifiers were used with a time constant of 0.03 sec and both incorporated top cut (i.e. low pass) filters giving activity 3 dB down above 10, 25, 50 and 75 Hz. Most recordings were made using the 75 Hz filter. Both amplifiers have six sensitivity levels, but the 50 μV/cm range was normally used. Some preparations exhibited higher voltage activity which required less amplification. The filter and sensitivity settings of the amplifiers were noted for each preparation, and a 50 μV d.c. calibrating voltage was recorded during each experiment so that brain activity levels could be calculated.

Devices indicated that the lowest frequency which could be integrated for electromyograph signals is 20 Hz. However reproducable quantitative results were obtained when olfactory lobe activity was integrated at frequencies of 7 - 10 Hz. Activity from the AC7C preamplifier was passed to the AC8 through a simple attenuator which was adjusted together with the gain control of the AC8 so that activity was recorded at the same level by both pens of the recorder and the AC8 was then set to integrate. Direct activity (from the AC7C) and integrated activity (from the AC8) were simulataneously recorded by the M2 pen recorder.

iv) The Stimulator:

The stimulator directed a stream of fluid through the single median dorsal nostril of the lamprey and into the nasal capsule where the liquid bathed the olfactory epithelium. The stimulator (Plate 10 and Figure 12) consisted of eight glass (Pyrex) tubes which were welded together and drawn to a fine point. This point was cut off, leaving a tube tapering to 5 mm outer diameter containing eight separate channels. A fine glass nozzle was then attached to the cut end with epoxy resin (Araldite).

The eight tubes were connected by polythene tubing to eight 100 ml. separating funnels, each containing a different solution. The system was filled by opening the tap of each funnel in turn, and allowing each tube to fill with its solution. When all eight tubes were full, the solutions could be turned on and off by simultaneously opening one tap and closing another. By this method a quick change of stimulants was effected when solutions were run at a rate of 12.5 ml per min.

After each experiment, the funnels, the tubing and the stimulator were thoroughly rinsed with distilled water. When necessary the whole stimulator system was washed in mild detergent and acetone both of which were thoroughly rinsed away with distilled water.

The volume of the stimulator nozzle was 0.015 ml. and therefore the time lag during stimulant change, was brief (ca. 0.1sec.) because stimulants were run at 12.5 ml. per min. into an olfactory sac whose average volume was 0.1 ml.

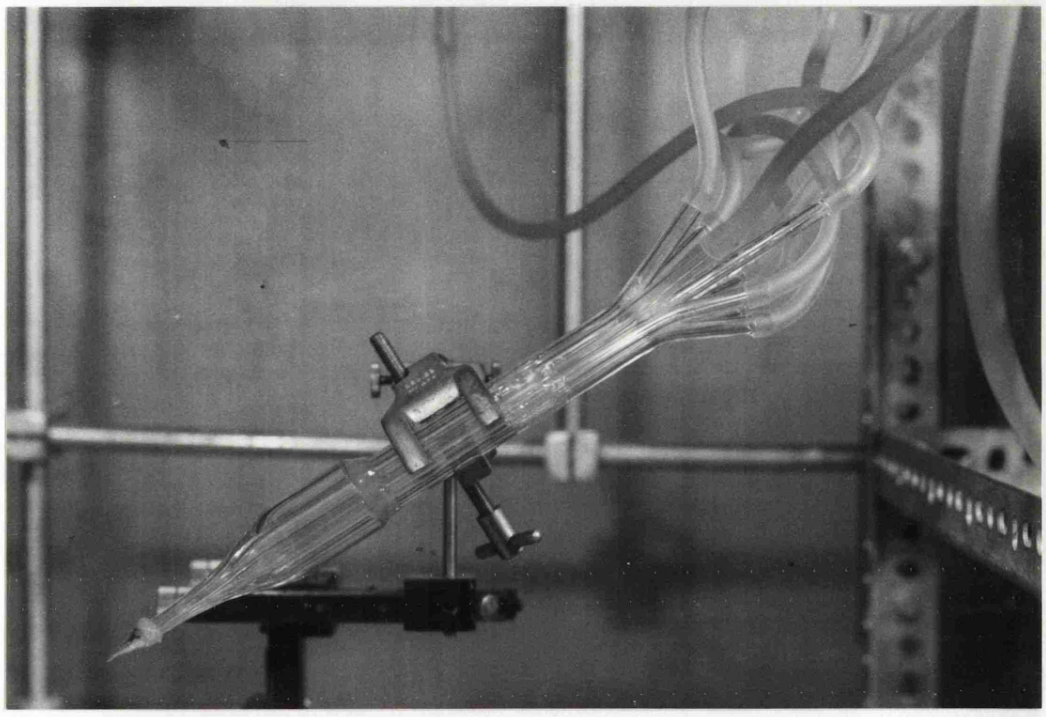
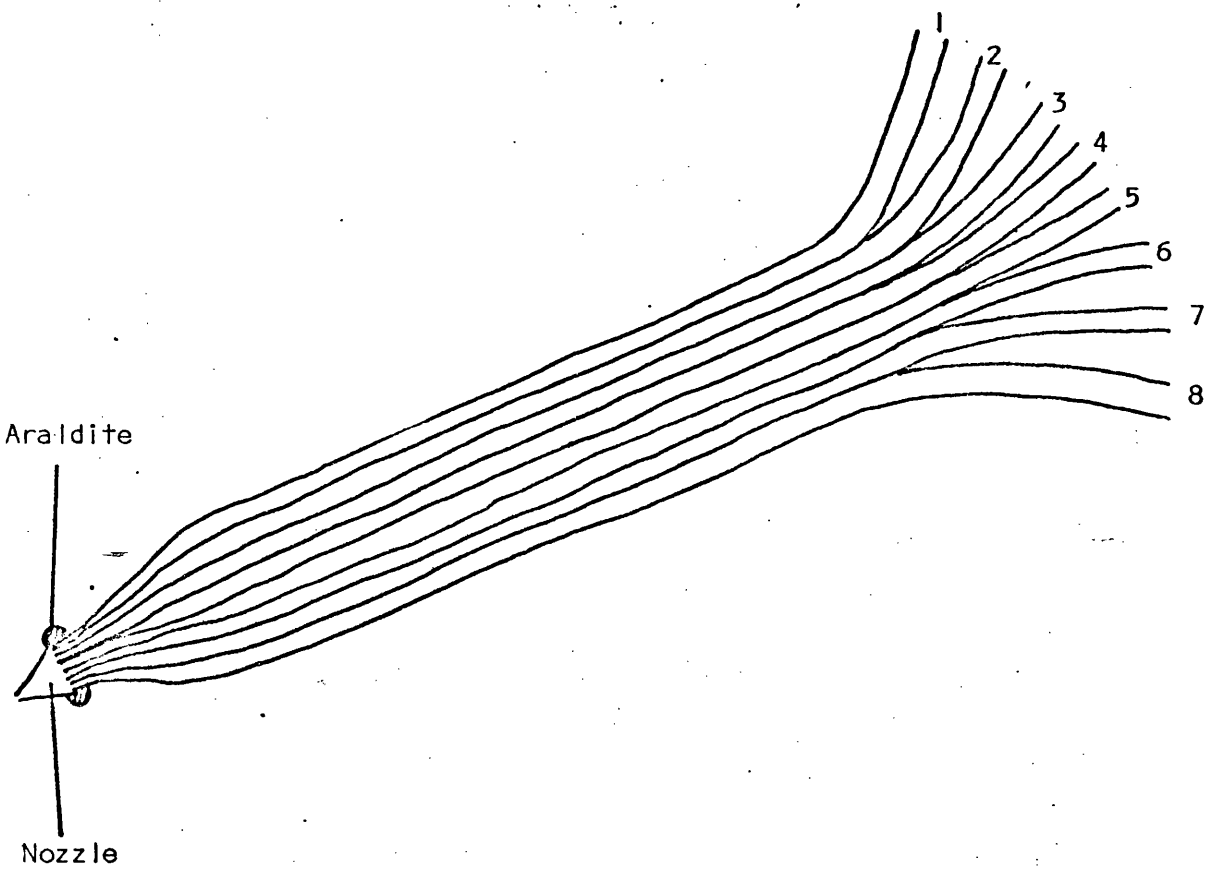


PLATE 10

Showing the stimulator on its micromanipulator support with attached polythene delivery tubes.

FIGURE 12 Diagrammatic view of stimulator construction



v) Maintenance of the Preparation:

Lampreys were immobilised by a mid-dorsal intramuscular injection (1 mgm/kgm body weight) of d-tubocurarine chloride (Sigma) dissolved in a ringer. The ringer composition was derived from an analysis of the blood of *L. fluviatilis* made by Galloway (1933) and consisted of the following chemicals made up to one litre with distilled water; 5.5g NaCl, 0.14g KCl, 0.12g $CaCl_2$ and 14 ml 0.1M $Na_2 HPO_4$ (pH 7.4). Each preparation was then positioned in an aluminium trough-like support as shown in Plate 9. Aerated pond water was run from an elevated reservoir at a rate of 30ℓ per hour through tubing to a nozzle in the wooden end-plate of the trough. The end-plate was cork-covered so that the lamprey sucker could be pinned over the nozzle. Water from the reservoir flowed through the lamprey's sucker, over and through the gills and out into the trough. Cotton wool was placed around and under each preparation so that only the olfactory apparatus and brain region were exposed above the water flowing through the trough. The temperature of the pond water in the reservoir was monitored during each experiment, and was found to increase by 1 - 2°C. By intermittently refilling the reservoir with fresh pond water, lamprey preparations set up as described could be maintained with strong heart beats for more than 3 hrs. Subcutaneous tissues exposed during the dissection were kept moist by occasionally brushing the tissues with cotton wool buds soaked in ringer.

vi) The Dissection and Experimental Procedure:

The brain was exposed under a Zeiss Epitechnoscope and Plates 11, 12, 13 and 14 show stages in the dissection. Plate 12 shows the exposed olfactory capsule after removal of the skin, and in Plate 13 the anterior epibranchial musculature has been removed. At this stage, the nozzle of the stimulator has been inserted into the nostril, and a small opening has been cut in the posterior dorsal cartilage of the capsule to allow stimulant fluids to escape. The recording electrode has been inserted through an incision in the cartilage covering the brain, and the indifferent electrode can be seen resting on surrounding musculature. After practice, very small incisions to permit the insertion of the electrode were made. Such incisions a) did not damage the underlying brain tissue, b) allowed the direct entry of the electrode tip onto the olfactory lobe, c) did not damage the choroid plexus overlying the brain and d) did not allow extraneous fluids to seep into the brain cavity.

Plate 14 shows the brain and electrode after the removal of the cartilage and the choroid plexus. Normally these tissues were not removed, but this photograph demonstrates the internal position of the electrode tip. Activity from other brain regions was recorded via other incisions through the cartilage. Any damage to the underlying choroid plexus resulted in copious bleeding and care was necessary when making incisions over the mid - and hind-brain regions.

Initially, olfactory lobe activity in response to stimulation by distilled water was recorded. The 50 μV d.c. calibrating voltage

PLATE 11

Anteriodorsal view of River lamprey showing top of lateral eye and pineal eye.

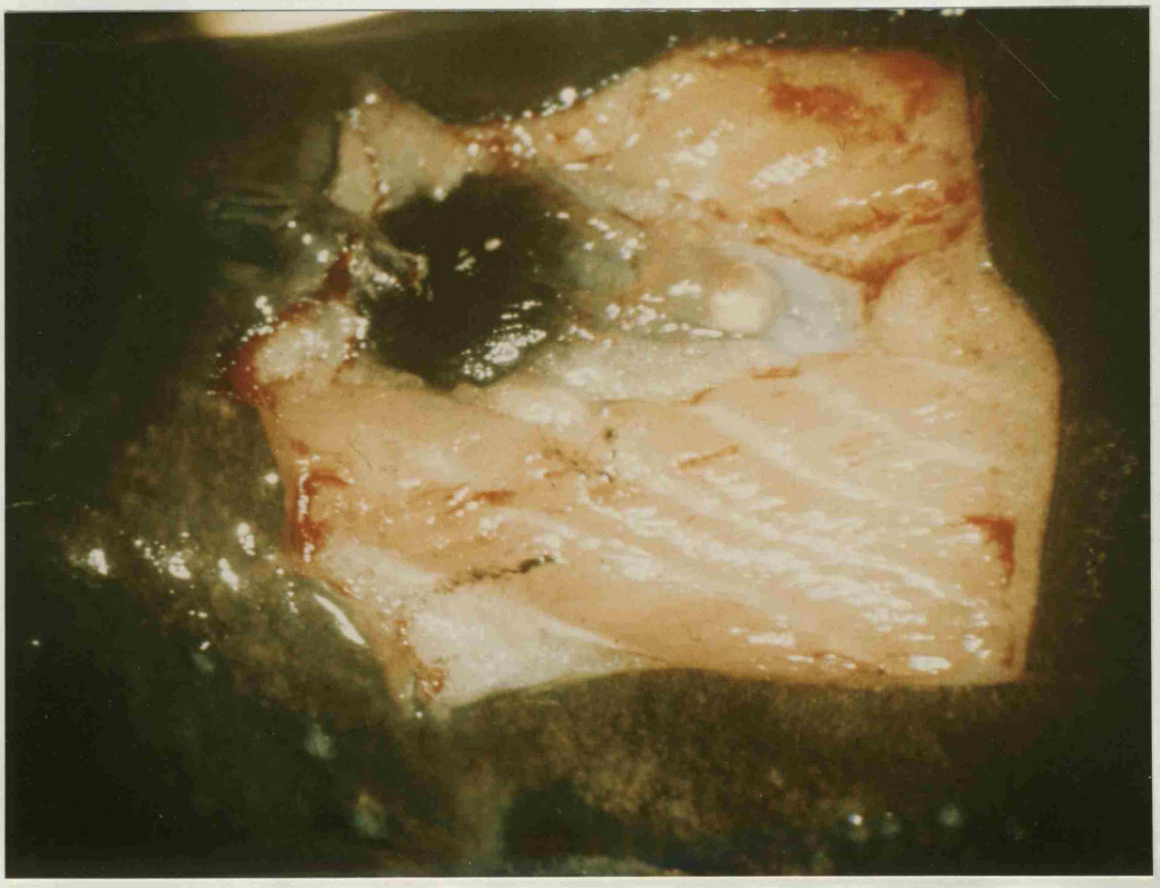


PLATE 12

Subcutaneous tissues exposed by removal of skin from nostril/pineal/ anterior brain region of a River lamprey. Nasal capsule evident as a round black organ behind nostril.

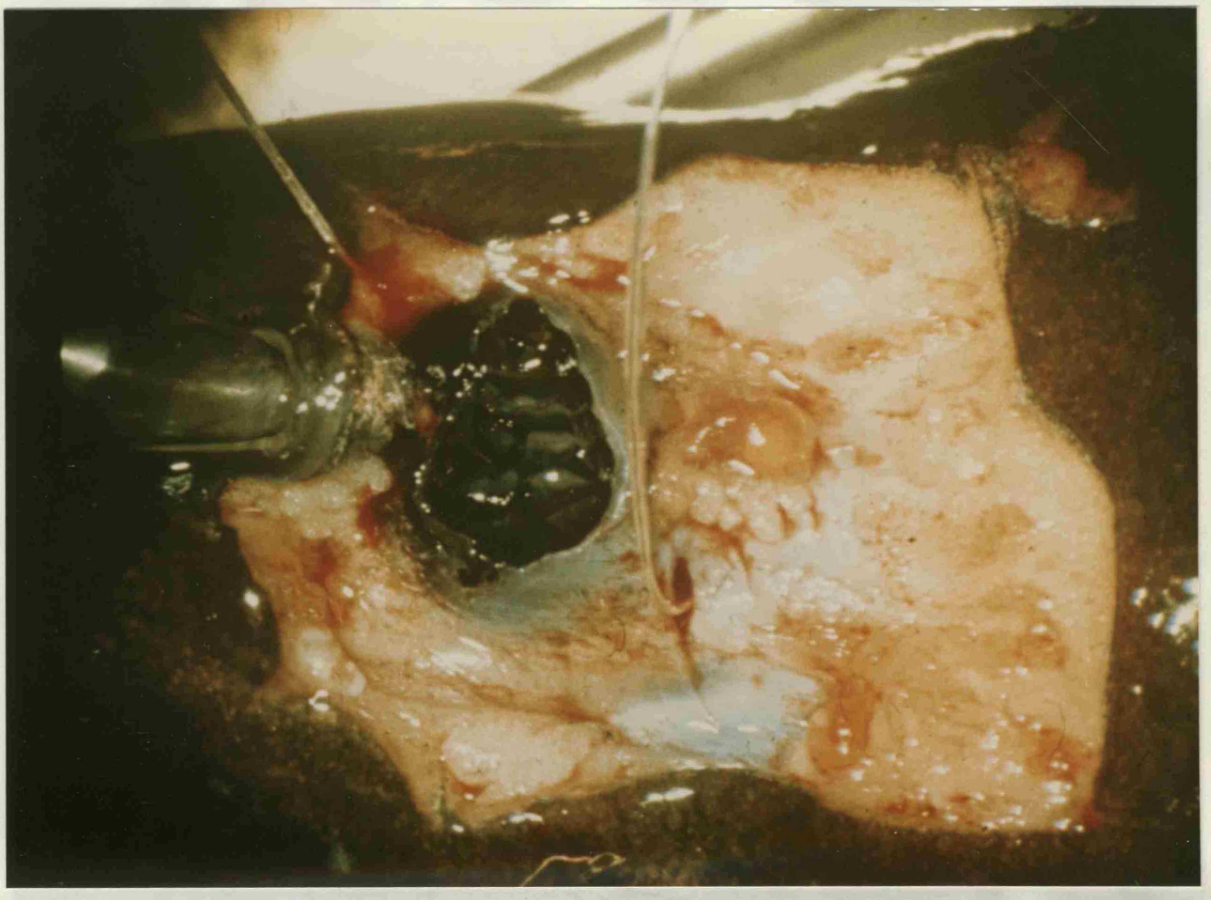


PLATE 13

River lamprey preparation set up for recording olfactory lobe responses to olfactory stimulation. Stimulator is in nostril, dorsal cartilage of nasal sac has been removed and electrode is *in situ* on brain surface through a small incision through cartilaginous brain covering.

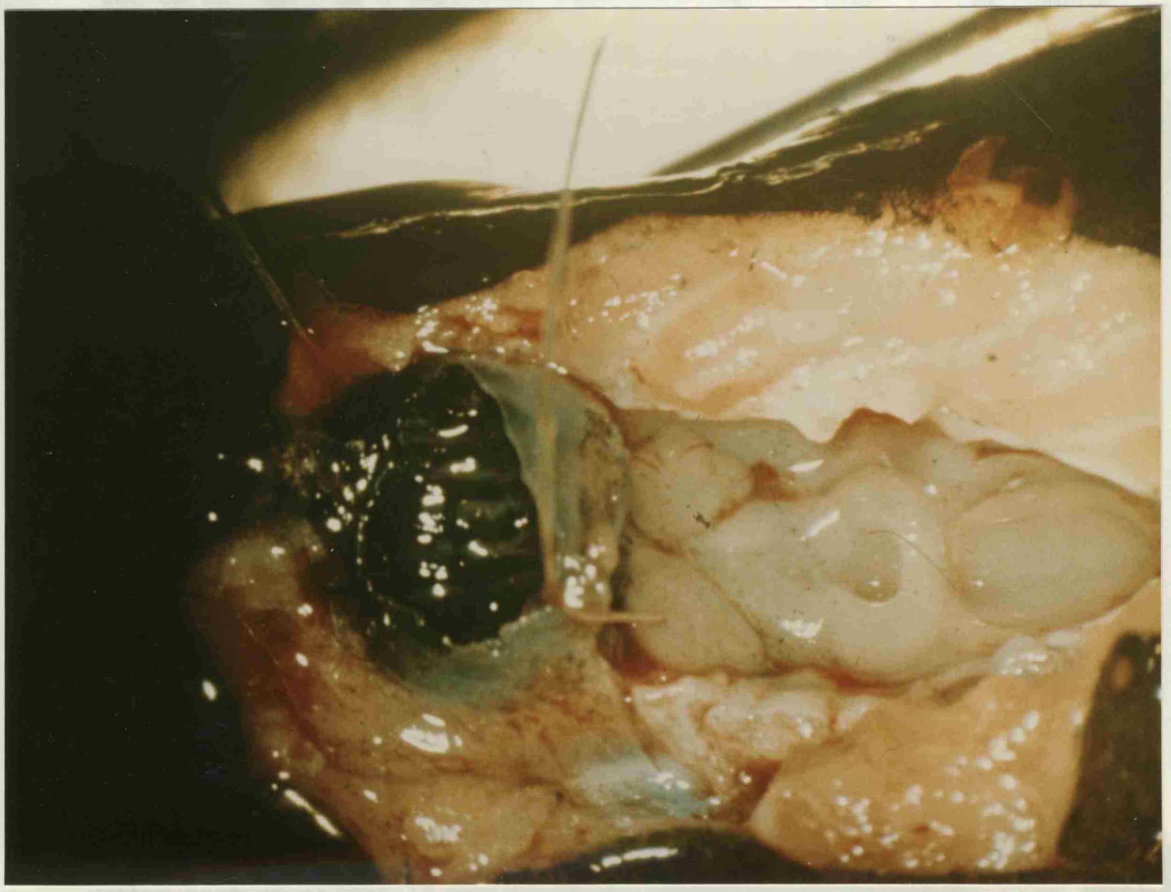


PLATE 14

River lamprey brain exposed to show electrode position during the recording of olfactory lobe responses. Choroid plexi removed to expose other brain regions.

was also recorded at this stage. This initial sequence was repeated at intervals during the experiment to monitor any changes in intrinsic activity or variations in the amplifying performance of the equipment. In all experiments, glass-distilled water from a single source was used as a reference stimulant for intrinsic activity and for comparison with activity evoked by other stimulant solutions.

The stimulation sequence consisted of a 45 sec wash with distilled water, followed by a 15 sec stimulation and a final 45 sec distilled water wash. This sequence was repeated using either the same or a different stimulant. The epithelium was therefore alternately washed and stimulated, and responses could be repeated or compared with responses evoked by other stimulants. In selected experiments the time of application of the stimulant was extended to 60 or 90 sec.

The stimulation sequence of the home water tests was that used by Ueda *et al* (1967) in work on salmon, and comprised washes with distilled water, non-home river water, distilled water and home river water each of either 15 sec or 40 sec duration.

At the end of all experiments, the stimulator was withdrawn from the nostril, and the electrode shanks were cut to leave the tip portion in situ on the brain. The length, weight and sex of the lamprey was then recorded. Some preparations were then severed at the first gill and the heads were fixed in Bouin's solution for 24 hr, before being preserved in 70% ethanol. These heads were later embedded and sectioned complete with electrode tips, so that after staining with Heidenheim's Azan stain (Pantin, 1964) microscopic examination permitted precise location of the electrode tip.

vii) Preparation of Stimulant Solutions:

In some experiments the following chemicals were used at known concentrations dissolved in distilled water; sodium chloride, mannitol, morpholine, hydrochloric acid, isoleucine methyl ester and 1,2-diaminoethane. In other experiments, solutions were all river water samples collected from various rivers. River water collected at a spawning site was designated the "home" river water for River lampreys caught at that site. Glass bottles, which had been washed in mild detergent and thoroughly rinsed several times with distilled water were used to collect samples, and the neck of the bottle was held upstream against the current during collection so that contamination from the hands did not enter the bottle. The precaution was taken in order to avoid chemicals released from mammalian skin, which have been shown to be repellant to some fish, (Idler *et al*, 1956; 1961). All water samples were used within 36 hrs of collection.

Solutions containing fish food odours were used in some experiments, as previous work (Shibuya, 1960; Huggins, 1968) has demonstrated that some commerical fish foods evoke responses in the olfactory epithelium of lampreys. An extract, made by filtering a suspension of a commercial fish food (Tetramin) in distilled water was used, as was a filtered suspension of macerated flesh from small Bass, 4 - 20 cm in length.

Solutions containing the odours of lamprey milt and ova were obtained by shaking these tissues, which had been stripped from sexually mature *L. fluviatilis* and *L. planeri*, in distilled water and filtering the suspension.

All suspensions were filtered through a fast, coarse filter paper (Greens 904), and if during any experiment, it was necessary to filter one solution, then all the solutions were filtered so that any contamination was present in all stimulants.

The electrophysiological results recorded in this thesis were based on 125 experiments, which included 27 experiments to show overall brain activity in ammocoetes(5 expts.), newly metamorphosed fluviatilis (5 expts.), adult marinus (6 expts.), and adult fluviatilis (11 expts.). The remaining 98 experiments were carried out in order to describe the olfactory lobe responses of adult fluviatilis to the following stimulants; river waters (26 expts.), 1.2-diaminoethane (28 expts.), isoleucine methyl ester(11 expts.), lamprey milt and eggs (10 expts.), sodium chloride(9 expts.), fish food(6 expts.), hydrochoric acid(5 expts.), and mascerated fish flesh(3 expts.).

C. RESULTS

i) Brain Activity in Lampreys:

Activity was recorded from the brains of ammocoetes *(Lampetra spp.)*, downstream migrant *L. fluviatilis*, upstream migrant *L. fluviatilis* and upstream migrant *P. marinus*. The four recording sites on the dorsal brain surface were:

i) the left olfactory lobe (telencephalon)

ii) the left optic tectum (mesencephalon)

iii) the medulla oblongata (myelencephalon)

and

iv) the anterior spinal cord.

Recordings were inspected for activity rates and amplitude, and optic tectum responses were evoked by directing the beam of a small flashlight onto the head region. The operation of the flashlight within the Faraday cage produced no spurious potentials in the activity records. The electrode recording sites, and the dominant frequencies and amplitudes of the recorded activity have been indicated with a diagrammatic lamprey brain (Figure 13).

Spontaneous electrical activity of the ammocoete brain (Figure 14) was greatest in the olfactory lobe where rhythmic activity at dominant frequencies of 3 - 4 Hz and amplitudes of 10 - 60 μV was recorded. The optic tectum, medulla and spinal cord exhibited little

Ammocoete (*Lampetra spps.*)	Downstream migrant (*L. fluviatilis*)	Upstream migrant (*L. fluviatilis*)	Upstream migrant (*P. marinus*)
3 - 4 Hz 10 - 60 µV	4 - 5 Hz 50 - 160 µV	4 - 9 Hz 40 - 100 µV	8 - 11 Hz 60 - 90 µV
-	8 - 12 Hz 10 - 20 µV	25 - 35 Hz 20 - 35 µV	22 - 26 Hz 25 - 30 µV
-	8 - 12 Hz 10 - 20 µV and spike potentials 35-60µV 3-4 Hz	Low amplitude background 10 - 20 µV and spike potentials 200-300µV 2-3Hz	Low amplitude background 10 - 20 µV and spike potentials 150-200µV 3Hz
-	Irregular 10 µV	Low level background activity 10 - 20 µV	Low level background activity 10 - 20 µV

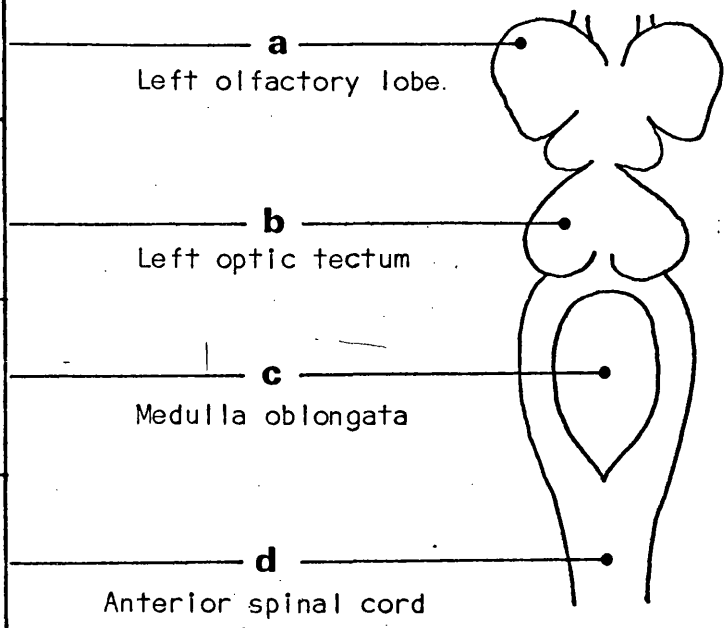


FIGURE 13 Diagrammatic lamprey brain showing four recording sites and the dominant frequencies and amplitudes recorded from four lamprey types.

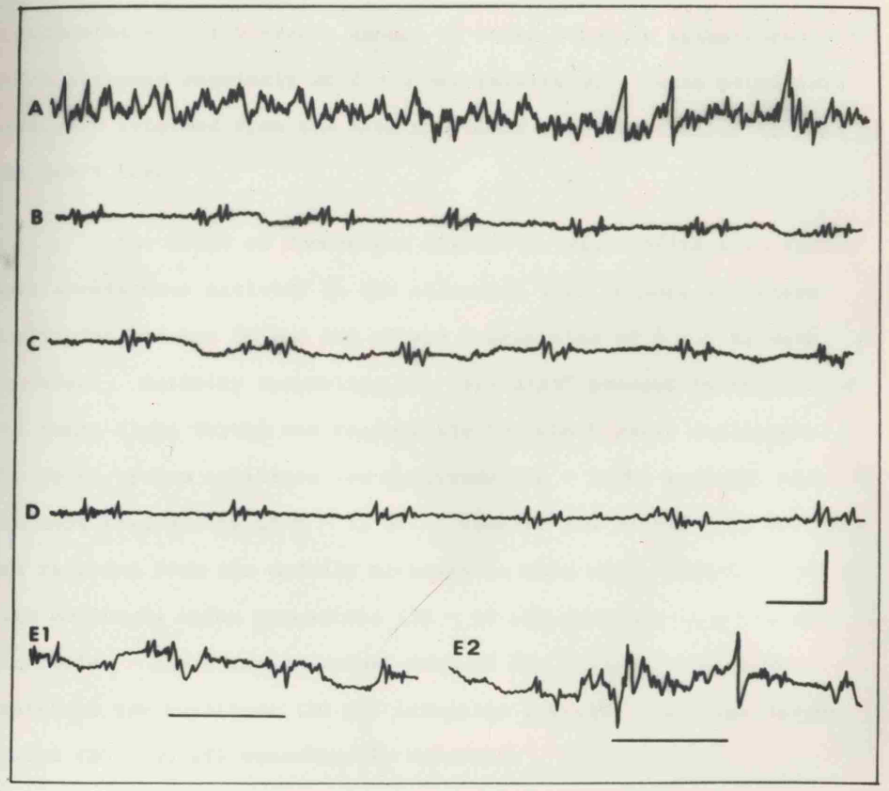


FIGURE 14

Montage of activity recorded from four sites on the brain of an ammocoete *(Lampetra spps.)*

Date of experiment	12.2.70
Length of ammocoete	107 mm
Temperature of experiment	13° C
Vertical calibration	50 µV
Horizontal calibration	1 sec

A Olfactory lobe activity

B Optic tectum activity

C Medulla oblongata activity

D Anteriodorsal spinal cord activity

E1 Response from optic tectum when flashlight beam directed onto the head of the ammocoete

E2 Response from optic tectum when flashlight beam directed onto the tail of ammocoete

(Duration of light stimulation indicated by horizontal line)

spontaneous activity except groups of three biphasic spike-potentials which occurred regularly at 2 - 3 sec intervals. These potentials were also recorded from the skin and their rate was similar to that of the heart beat.

The brain of downstream migrant *L. fluviatilis* also showed most spontaneous activity in the olfactory lobe (Figure 15) where amplitudes between 50 and 160 μV and frequencies of 4 - 5 Hz were recorded. Activity resembling the "spindles" present in records of the human alpha rhythm was responsible for the highest amplitudes. The optic tectum exhibited low amplitude (10 - 20μV) activity with dominant frequencies at 8 - 12 Hz. Similar low level, fast activity was recorded from the medulla although in this case, trains of 3 - 5 high amplitude spike potentials (35 - 60 μV) occurred at 3 - 4 sec intervals. The anterior spinal cord of the downstream migrant exhibited low amplitude (10 μV) irregular activity, although larger spikes (20 - 40 μV) occasionally occurred.

The olfactory lobe activity of upstream migrant *L. fluviatilis* (Figure 16) was characterised by dominant frequencies of 5 - 9 Hz at amplitudes of 40 - 100 μV, and spindle-like components were commonly involved. Optic tectum activity was faster at frequencies of 25 - 35 Hz with amplitudes of 20 - 35 μV. Experiments were only carried out in a brightly-lit laboratory, and this situation, which evokes similar optic tectum activity in other fish has been termed an "arousal" condition (Enger, 1957; Schadé and Weiler, 1959). The medulla oblongata of upstream migrants consistently exhibited low amplitude (10 - 20 μV) background activity, the dominant frequencies of which were high and difficult to assess because regular, high amplitude (200 - 300 μV)

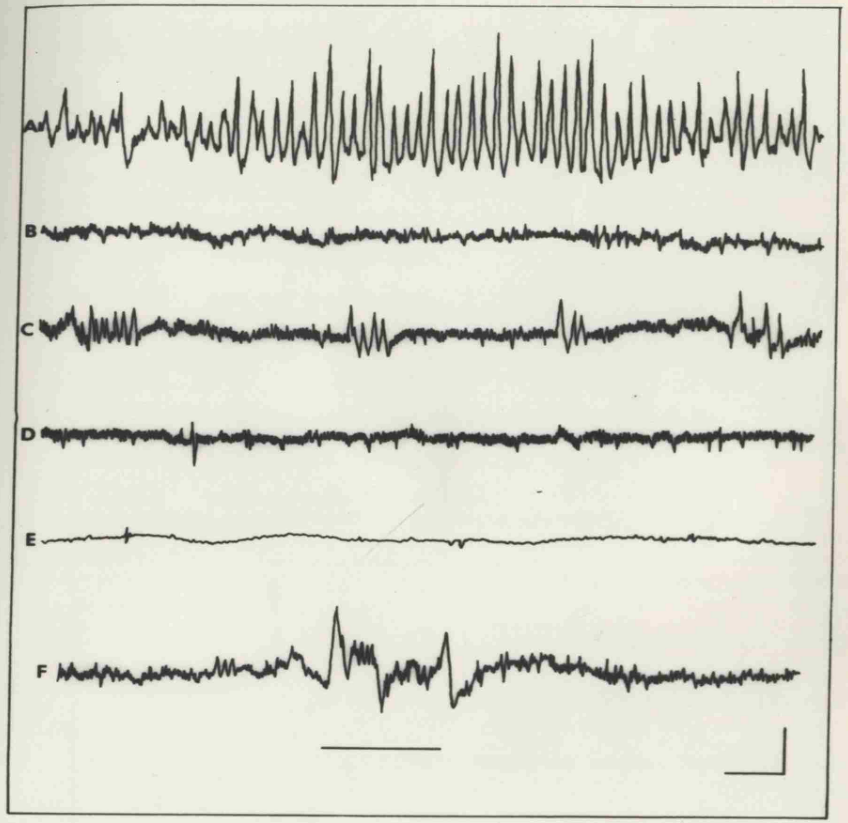


FIGURE 15

Montage of activity recorded from four sites on the brain of a downstream migrant River lamprey *(L. fluviatilis)*

Date of experiment	12.2.70
Length of lamprey	95 mm
Weight of lamprey	1.35 gm
Temperature of experiment	13° C
Vertical calibration	50 µV
Horizontal calibration	1 sec

A Olfactory lobe activity

B Optic tectum activity

C Medulla oblongata activity

D Anteriodorsal spinal cord activity

E Activity recorded when electrode placed on dorsal musculature

F Response of left optic tectum when flashlight beam directed onto the left eye of lamprey

(Duration of light stimulus indicated by horizontal line)

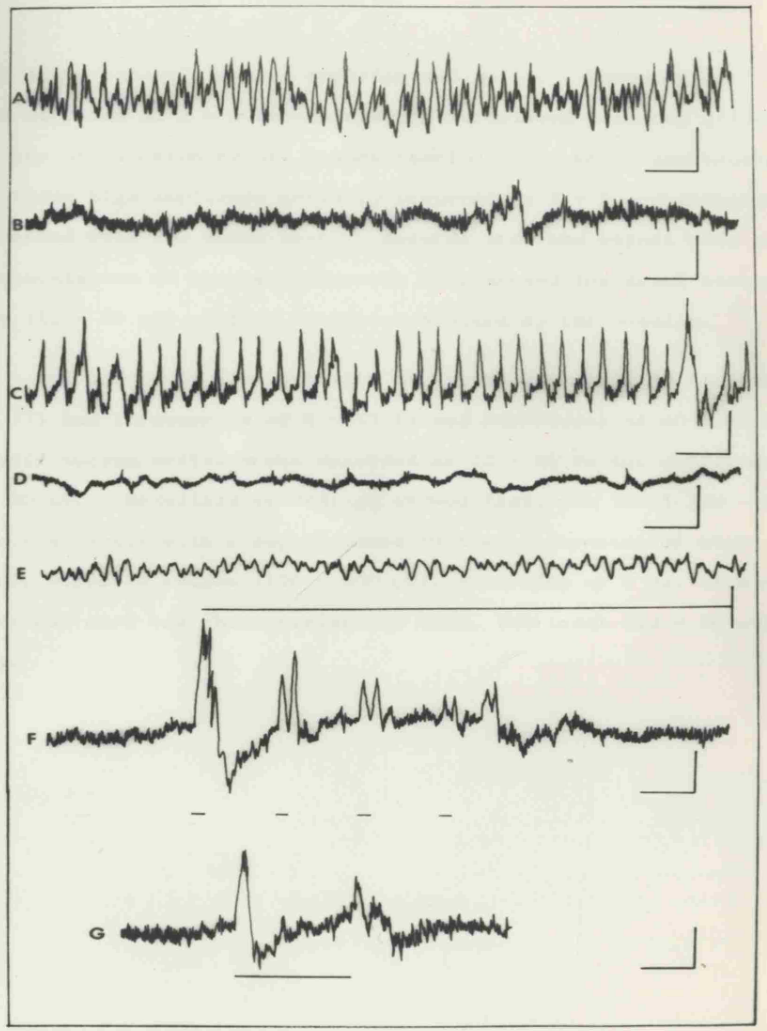


FIGURE 16

Montage of activity recorded from four sites on the brain of an upstream migrant River lamprey *(L. fluviatilis)*

Date of experiment	19.2.71
Length of lamprey	34.8 cm
Weight of lamprey	65.6 gm
Sex	Female
Temperature of experiment	10° C
Vertical calibration	50 µV
Horizontal calibration	1 sec

A Olfactory lobe activity

B Optic tectum activity

C Medulla oblongata activity

D Anteriodorsal spinal cord activity

E Optic tectum activity

F Response of left optic tectum to four 200 msec light flashes directed onto left eye of lamprey

G Response of left optic tectum to one 2.2 sec photic stimulation directed onto left eye of lamprey

(Duration of light stimuli indicated by horizontal line)

biphasic spike-potentials were superimposed on it. These spikes occurred at rates of 2 - 3 Hz and closely correlated with the gill-beat rate of the preparation before immobilization. Further disturbances in this rhythmic high amplitude activity occurred at 3 - 5 sec intervals and coincided with the heart beat. Records from the spinal cord and dorsal musculature of upstream migrants both showed low level background activity (10 - 20 μV) similar to that exhibited by the medulla.

The olfactory lobe activity of upstream migrant *P. marinus* (Figure 17) had frequencies of 8 - 11 Hz and amplitudes of 60 - 90 μV, while optic tectum activity was recorded at 22 - 26 Hz and amplitudes of 25 - 30 μV. Medullary recordings showed fast, low level (10 - 20 μV) background activity with a superimposed rhythmic component of high amplitude, biphasic spikes (150 - 200 μV), occurring at 2 Hz. Activity of the spinal cord was characterised by fast, low level (10 - 20 μV) activity.

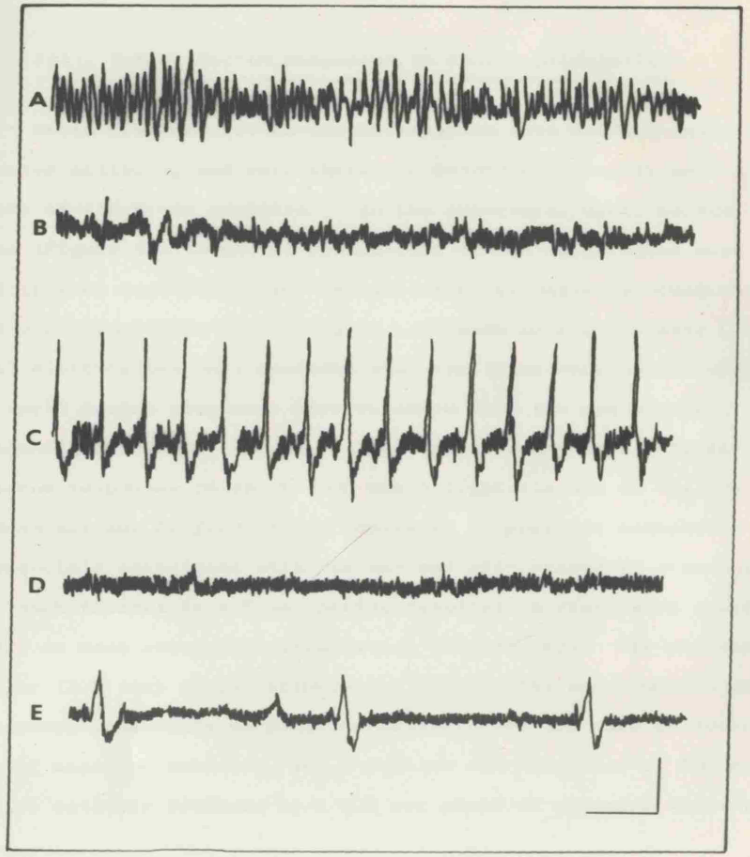


FIGURE 17

Montage of activity recorded from sites on the brain of an upstream migrant Sea lamprey *(P. marinus)*

Date of experiment	8.6.70
Length of lamprey	72.5 cm
Weight of lamprey	678 gm
Sex	Male (sexually immature)
Temperature of experiment	19.5° C
Vertical calibration	50 µV
Horizontal calibration	1 sec

A Olfactory lobe activity

B Optic tectum activity

C Medulla oblongata activity

D Anteriodorsal spinal cord activity

E Activity recorded when electrode placed on mid-dorsal skin showing electrocardiographic components.

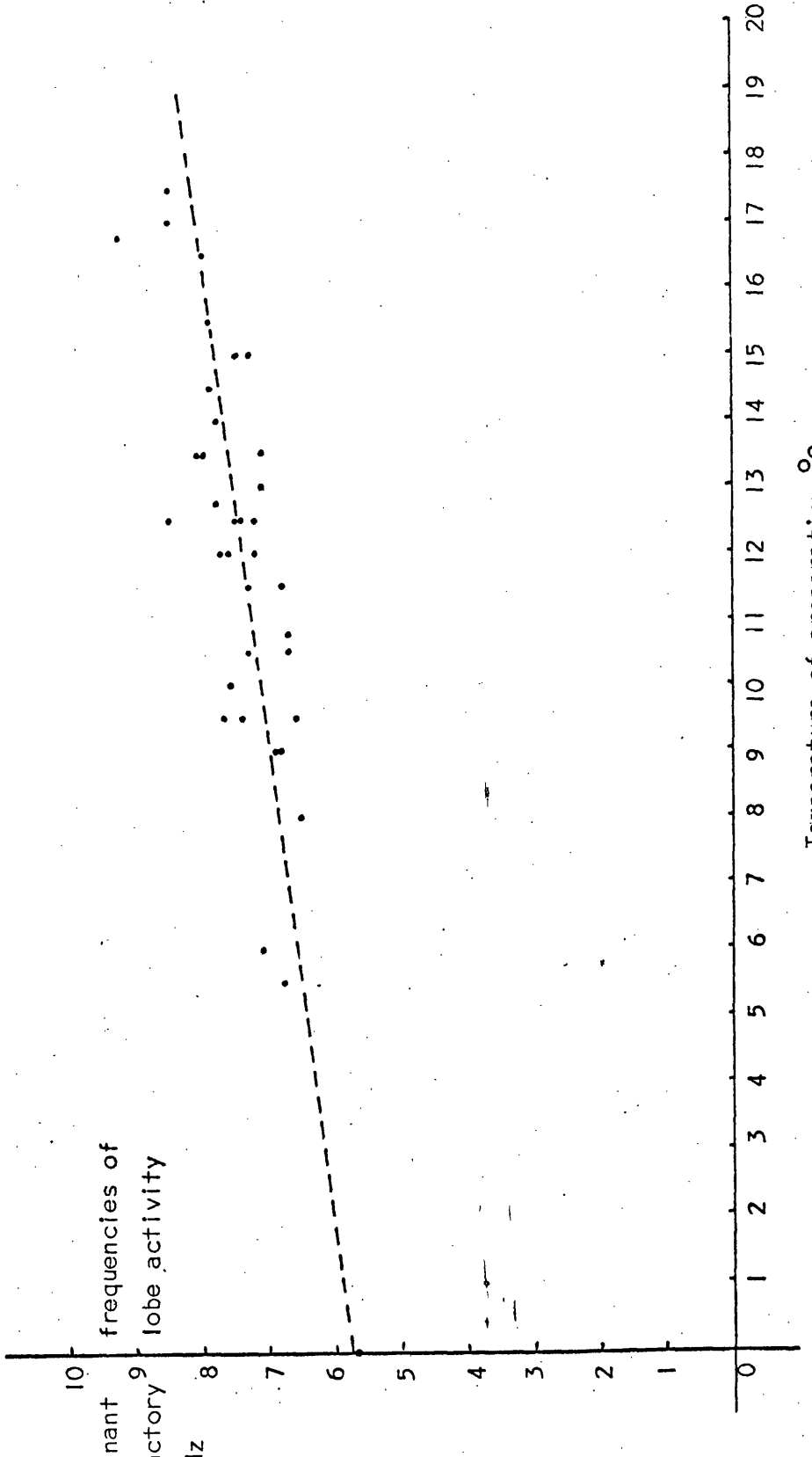
ii) Optic Tectum Responses to Photic Stimulation:

Brain responses to photic stimulation were evident only in optic tectum activity, and were therefore recorded primarily as indicators of electrode position. In the ammocoete, optic tectum responses (Figure 14) to photic stimulation of the tail region were often of greater amplitudes (60 - 80 μV) than responses recorded when the head was illuminated (25 - 35 μV). In both cases, diphasic potential disturbances were recorded when the light went on and off. Similar optic tectum responses were recorded when the eye of the downstream migrant River lamprey was photically stimulated (Figure 15). Optic tectum responses to short (200 msec) light flashes on the eye of upstream migrant *L. fluviatilis* consisted of positive monophasic spike-potentials coincident with the on- and off- phases of stimulation and four such flashes in a 5 sec period resulted in diminished on/off potentials at each subsequent stimulation (Figure 16). The response to a longer (2.2 sec) photic stimulation (Figure 16) was composed of an on-response consisting of positive activity for 300 msec followed by 1 sec of negative activity, and a smaller off- response of 300 msec of positive activity followed by a 0.5 sec phase of negative activity.

iii) Olfactory Lobe Activity and Temperature:

Records were kept of the dominant frequencies of olfactory lobe activity together with the temperature of preparations. It was found that the temperature of pond water used for maintenance of preparations varied between 0.0° C and 17.5° C through the autumn-spring period when lampreys were available. Data from 37 preparations used during this period (Figure 18) showed a linear correlation ($p < 0.001$) between frequency and temperature, described by the formula $y = 0.14x + 5.77$. The Q_{10} ($0 - 10^{\circ}$ C) for the frequency variation was 1.24.

FIGURE 18 Dominant olfactory lobe activity frequencies and temperatures of 37 preparations used during the Autumn-Spring period.



iv) Olfactory Lobe Activity and Respiration:

Olfactory lobe activity was recorded from some preparations which were allowed to recover from the immobilising effects of d-tubocurarine chloride. Slow, diphasic potential changes, coincident with muscular contractions of gill beat, became increasingly evident (Figure 19) until eventually only very high amplitude (200 - 300 μV) spike-like potentials were recorded. These potentials have been recorded from the water surrounding the head region of unanaesthetized lampreys (Kleerekoper and Sibakin, 1956a; b; 1957).

Arrested gill perfusion resulted in a gradual reduction in the amplitude of olfactory lobe activity, but following recommencement of perfusion, activity quickly returned to original levels (Figure 20).

Fig. 15A shows olfactory lobe activity from a curarised river lamprey, and Fig. 19 shows three stages in the appearance of slow (2-3 Hz) disphasic potential changes in olfactory lobe activity recorded during recovery from immobilisation.

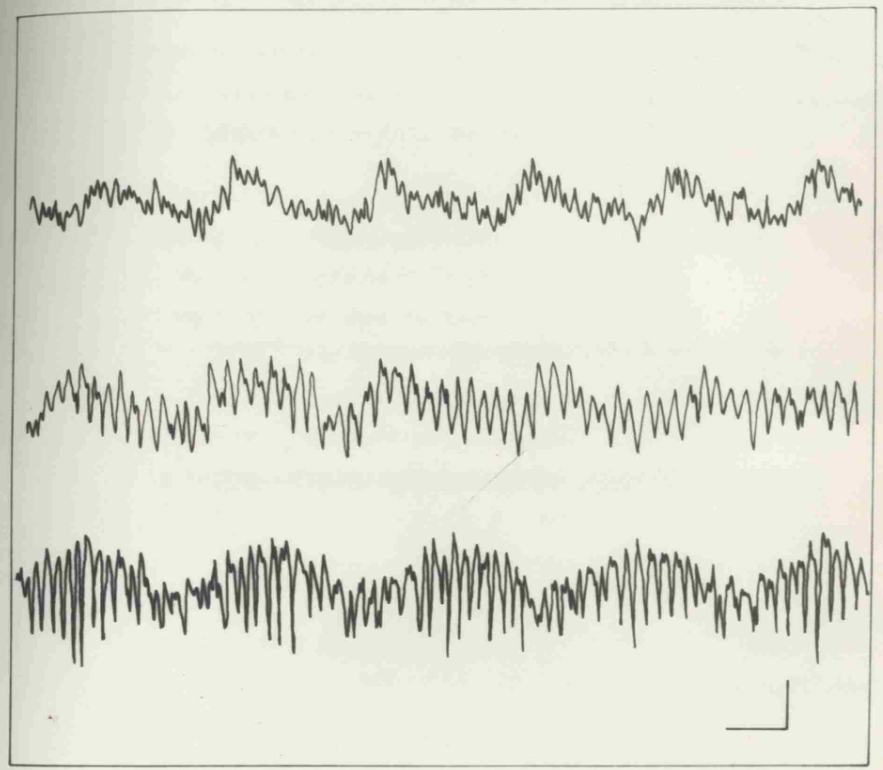


FIGURE 19

Montage of activity recorded from the olfactory lobe of an upstream migrant River lamprey

Date of experiment	13.1.71
Length of lamprey	33.0 cm
Weight of lamprey	56.7 gm
Sex	Male
Temperature of experiment	8.5° C
Vertical calibration	50 µV
Horizontal calibration	1 sec

Three traces of olfactory lobe activity recorded from a preparation which was allowed to recover from the immobilising effects of d-tubocurarine chloride.

Upper trace: Olfactory lobe activity

Middle trace: Olfactory lobe activity in response to stimulation of olfactory epithelium with 100 ppm solution of 1,2-diaminoethane

Lower trace: Olfactory lobe activity in response to stimulation of olfactory epithelium with 1000 ppm solution of 1,2-diaminoethane.

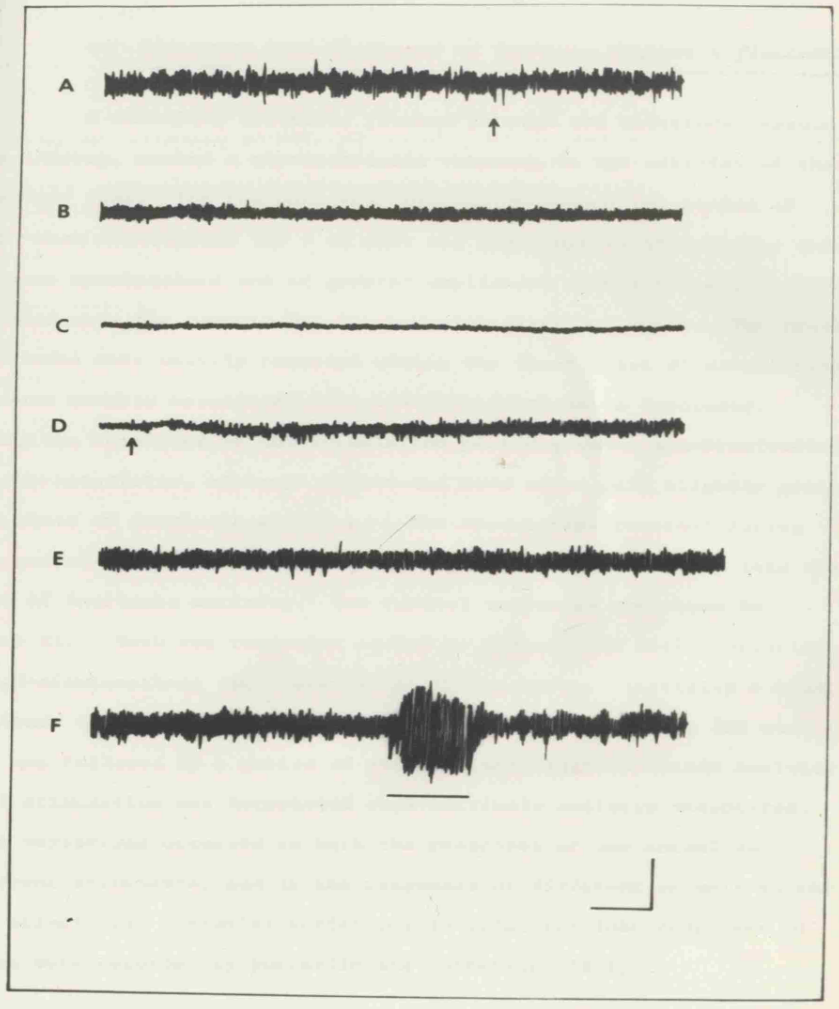


FIGURE 20

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing effects of arrested and recommenced gill perfusion.

Date of experiment	28.10.70
Length of lamprey	31.7 cm
Weight of lamprey	56.2 gm
Sex	Male
Temperature of experiment	7° C
Horizontal calibration	10 sec
Vertical calibration	100 µV

A Gill perfusion terminated at arrow

B Activity recorded 30 min after gill perfusion terminated

C Activity recorded 45 min after gill perfusion terminated

D Activity recorded 46 min after gill perfusion terminated, recommencement of perfusion at arrow

E Activity recorded 1 min after gill perfusion recommenced

F Activity recorded 5 min after gill perfusion recommenced showing response to stimulation of olfactory epithelium with 300 ppm solution of 1,2-diaminoethane.

v) Olfactory Lobe Responses of Upstream Migrant *L. fluviatilis*:

A stimulant solution, flushed through the olfactory capsule of a lamprey, evoked a characteristic response in the activity of the olfactory lobe. The response was present throughout the period of short-term stimulation (15 - 45 sec) and was composed of activity which was more synchronised and of greater amplitudes than intrinsic activity recorded when the capsule was flushed with distilled water. The greatest amplitudes were usually recorded within the first 5 sec of stimulation, and were usually associated with a slight increase in frequency. During the remainder of the stimulation period, greater synchronisation was characteristic, although amplitudes were often only slightly greater than those of intrinsic activity. The frequencies recorded during this period of the response were usually the same or slightly less than those of intrinsic activity. Two typical responses are shown in Figure 21. Both are responses evoked by stimulation with a solution of 1,2-diaminoethane (1000 ppm) in distilled water. Initially a high amplitude (200 - 300 μV) diphasic spike was evoked, lasting 300 msec. This was followed by a period of synchronised, high amplitude activity until stimulation was terminated when intrinsic activity reappeared. Minor variations occurred in both the responses of one animal to different stimulants, and in the responses of different animals to the same stimulants. Similar variations in olfactory lobe responses of salmon were recorded by Sutterlin and Sutterlin (1971).

Although strong olfactory lobe responses could be evoked by stimulating the epithelium with solutions prepared by filtering suspensions of fish food (Tetramin) or mascerated fish flesh in distilled water (Figure 22), these stimulants were chemically complex and

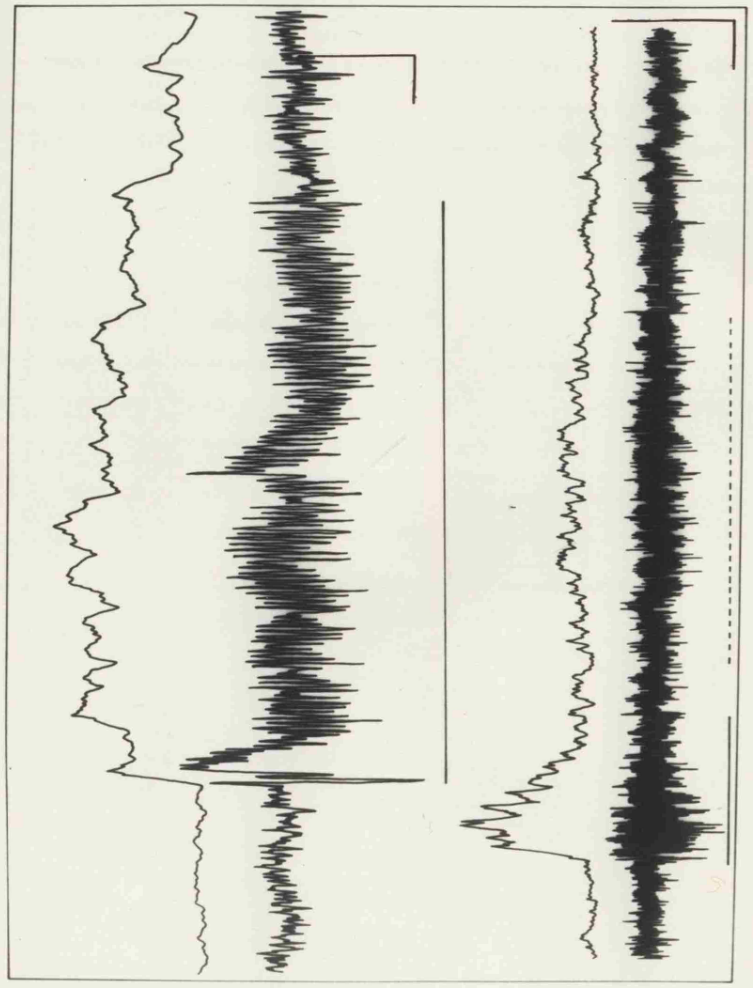


FIGURE 21

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing two responses to stimulation of the olfactory epithelium with a stimulant solution of 1,2-diaminoethane (1000 ppm) in distilled water.

Date of experiment	30.11.70
Length of lamprey	34.3 cm
Weight of lamprey	77.7 gm
Sex	Male
Temperature of experiment	8° C
Upper trace : Horizontal calibration	1 sec
Vertical calibration	100 µV
Lower trace : Horizontal calibration	5 sec
Vertical calibration	100 µV

A faint after-response, indicated by a dashed line under the lower trace, is evident and responses of this type were further investigated and are reported later.

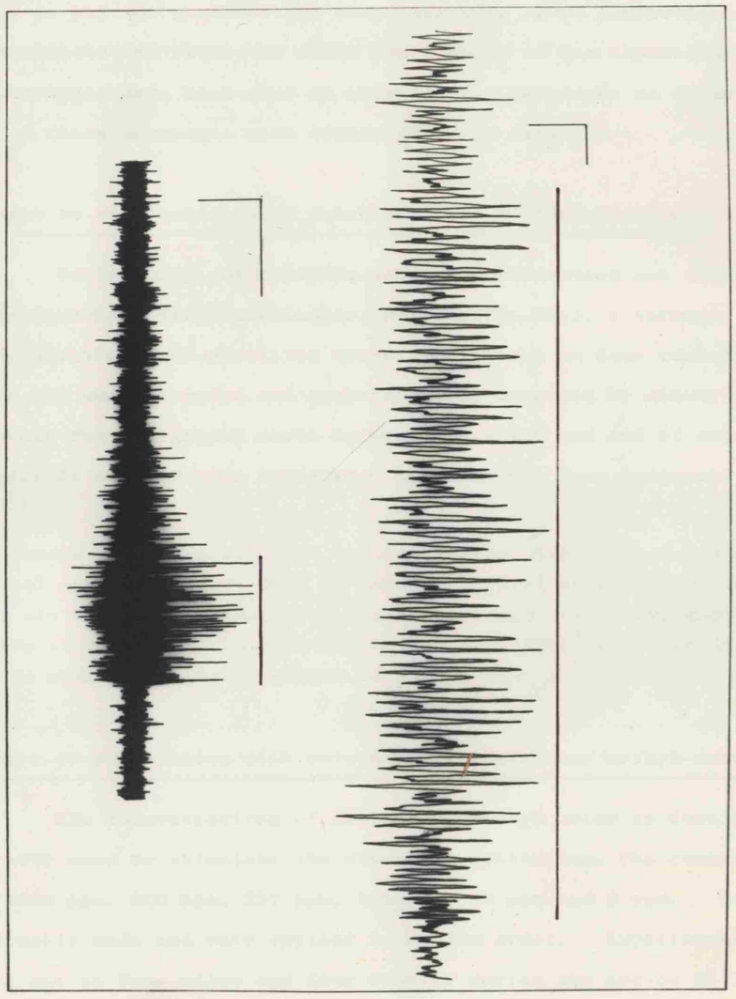


FIGURE 22

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing responses to stimulation with filtered solutions of fish food (upper trace) and mascerated fish flesh (bottom trace), in distilled water.

Date of experiment	12.11.70
Length of lamprey	33.4 cm
Weight of lamprey	83.7 gm
Sex	Female
Temperature of experiment	7° C
Upper trace : Horizontal calibration	10 sec
Vertical calibration	50 µV
Bottom trace : Horizontal calibration	1 sec
Vertical calibration	50 µV

quantitative assessments of responses were not possible. It was therefore decided to attempt quantitative responses with known concentrations of pure chemicals and Figure 23 shows the results of one experiment in which five chemicals have been used as stimulants. Responses to solutions of three of these chemicals were investigated in detail.

Responses to stimulation with solutions of 1,2-diaminoethane:

Preliminary quantitative studies were carried out using 1,2-diaminoethane (ethylenediamine, $NH_2.CH_2.CH_2.NH_2$), a strongly odourous liquid, miscible with distilled water. Responses to four concentrations (Figure 24) were recorded and quantitatively assessed by measuring the area under the integrator curve between the start and end of stimulation. In Figure 24 the 1000 ppm integrated response has been indicated by

Responses were also recorded by stimulating with concentrations of mannitol and morpholine, both of which were available in pure form. Lampreys are unlikely to encounter these chemicals naturally, and morpholine is especially interesting as Hasler (1966) has shown it to be neither attractive nor repellant to fish.

Responses to stimulation with solutions of isoleucine methyl ester:

Six concentrations of isoleucine methyl ester in distilled water were used to stimulate the olfactory epithelium, the concentrations being 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 50 ppm and 5 ppm. Solutions were freshly made and were applied in random order. Experiments were carried out on four males and four females during the period 26.2.71 to 24.3.71, at temperatures between 9.5 and 12.0°C. Figures 26 and 27 show responses which were recorded during one of the experiments.

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Responses to stimulation with solutions of isoleucine methyl ester:

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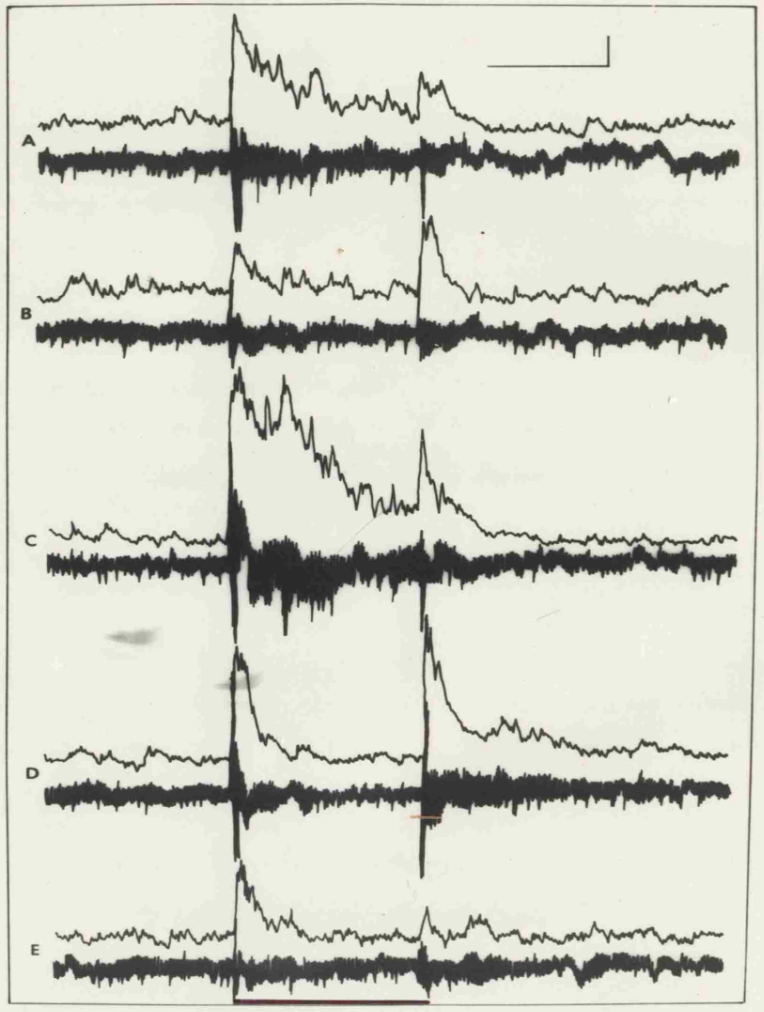


FIGURE 23

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing responses to stimulation of the olfactory epithelium with distilled water solutions of five chemicals.

Date of experiment	30.12.70
Length of lamprey	33.9 cm
Weight of lamprey	69.2 gm
Sex	Male
Temperature of experiment	10° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

A 3000 ppm solution of 1,2-diaminoethane

B 6000 ppm solution of mannitol

C 3000 ppm solution of NaCl

D 3000 ppm solution of isoleucine methyl ester

E 3000 ppm solution of morpholine.

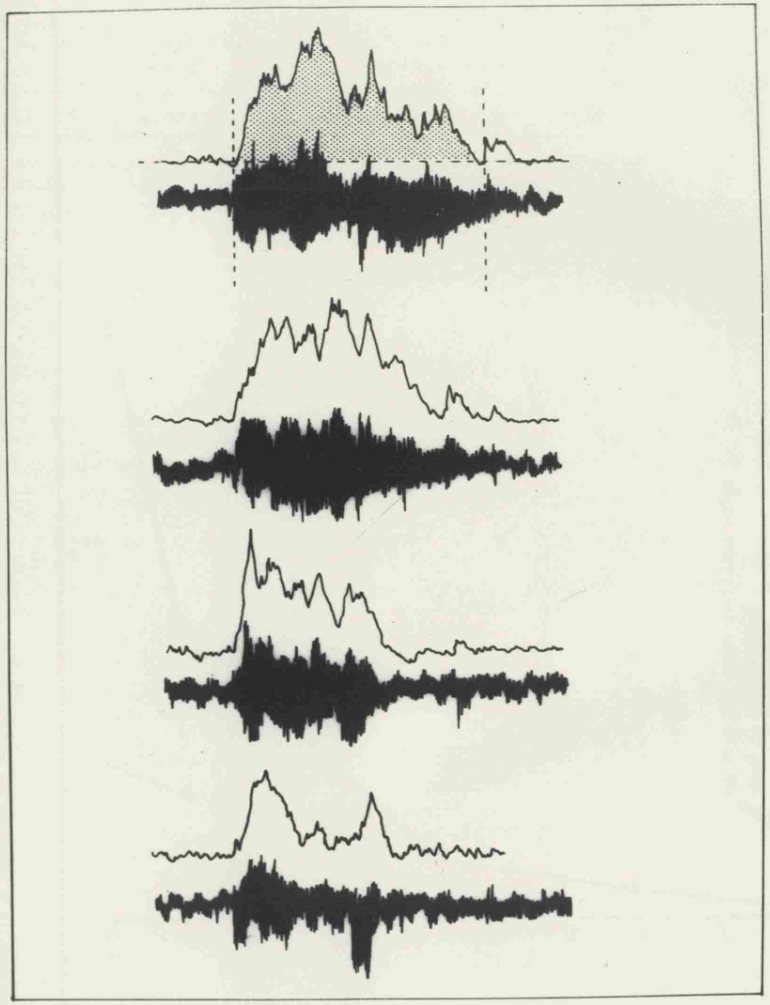


FIGURE 24

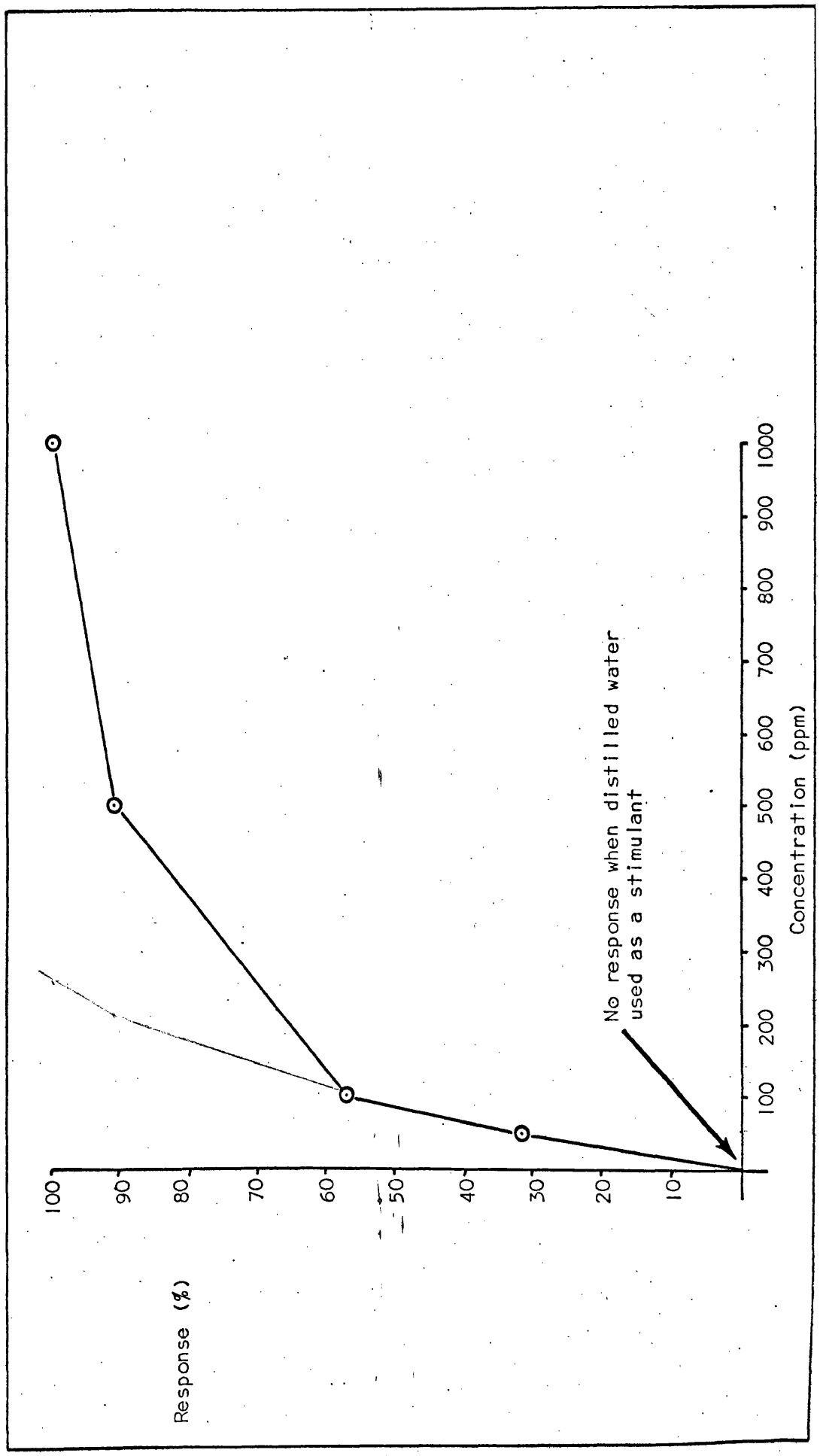
Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing responses to stimulation of the olfactory epithelium with distilled water solutions of 1,2-diaminoethane at four concentrations.

Date of experiment	23.11.70
Length of lamprey	36.2 cm
Weight of lamprey	75.2 gm
Sex	Female
Temperature of experiment	9° C
Horizontal calibration	50 µV
Vertical calibration	10 sec

Upper trace: 1000 ppm solution of 1,2-diaminoethane
Second trace: 500 ppm solution
Third trace: 100 ppm solution
Bottom trace: 50 ppm

In the upper trace the activity evoked by epithelial stimulation and indicated by the integrator trace has been assessed and is shown by hatching.

FIGURE 25 Assessed integrator responses (see Figure 24) expressed as percentages of the assessed integrator response to a solution of 1000 ppm 1,2-diaminoethane in distilled water.



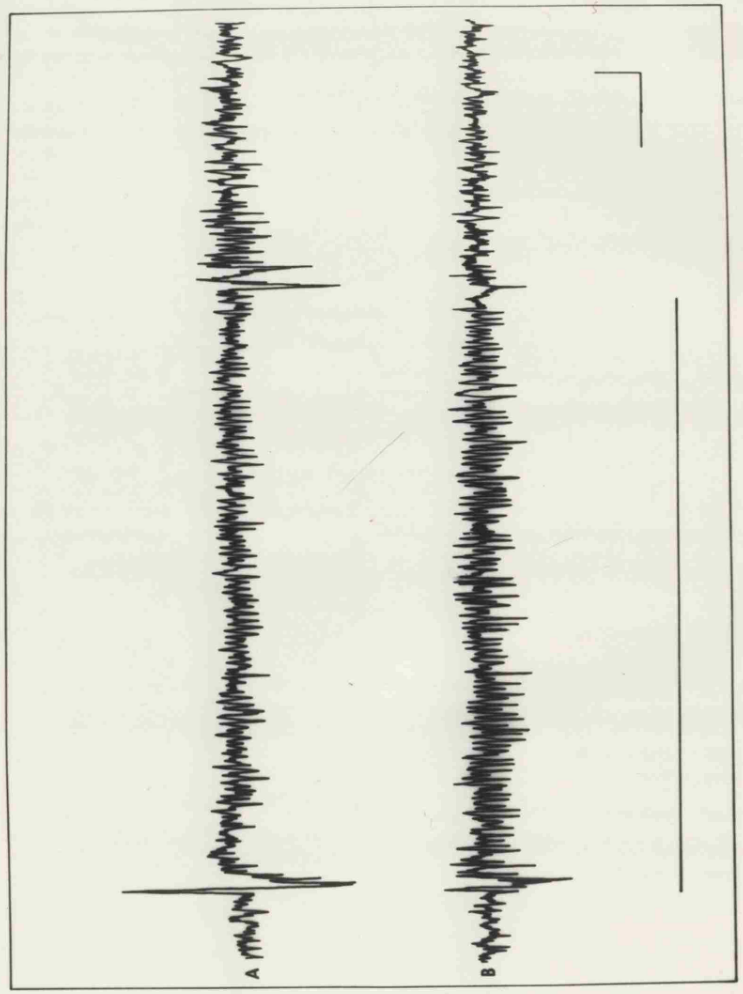


FIGURE 26

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey showing responses to stimulation of the olfactory epithelium with distilled water solutions of isoleucine methyl ester.

Date of experiment	1.3.71
Length of lamprey	31.5 cm
Weight of lamprey	58.8 gm
Sex	Female
Temperature of experiment	9.5° C
Horizontal calibration	1 sec
Vertical calibration	50 µV

A 1000 ppm solution of isoleucine methyl ester

B 250 ppm solution of isoleucine methyl ester

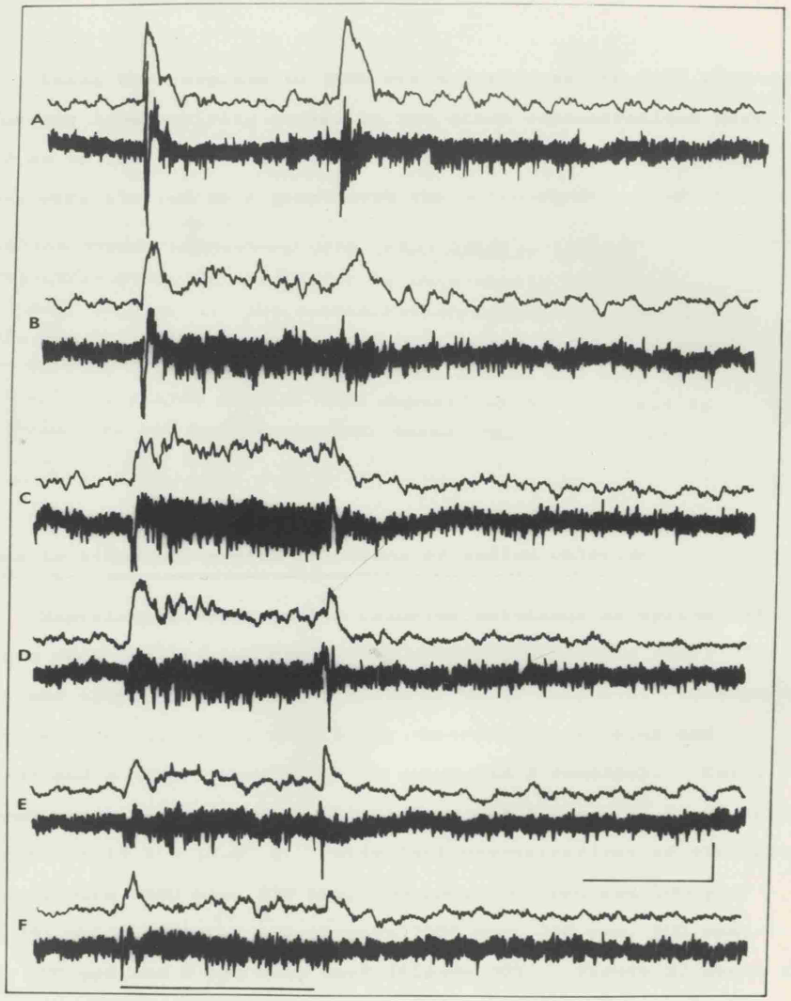


FIGURE 27

Montage of olfactory lobe activity recorded from an upsteam migrant River lamprey showing responses to stimulation of the olfactory epithelium with distilled water solutions of isoleucine methyl ester.

Date of experiment	1.3.71
Length of lamprey	31.5 cm
Weight of lamprey	58.8 gm
Sex	Female
Temperature of experiment	9.5° C
Horizontal calibration	1 sec
Vertical calibration	50 µV

A 1000 ppm solution of isoleucine methyl ester

B 500 ppm solution

C 250 ppm solution

D 125 ppm solution

E 50 ppm solution

F 5 ppm solution

Using the response to 1000 ppm solution as the 100% response, the olfactory lobe activity evoked by the other concentrations were assessed as in the 1,2-diaminoethane experiments. Mean percentage responses were plotted on a graph with the standard errors of the

To allow direct comparison with other similar studies, the concentration/response data reported in this thesis have been arithmetically displayed. The concentration/response curve shown in Fig 25. can be linearly transformed by a logarithmic function, but other curves, e.g. Fig. 28, show finite maxima and have been arithmetically displayed so that this aspect can be discussed in neurophysiological and neuroanatomical terms (pages 161 -164).

Responses to stimulation with solutions of sodium chloride:

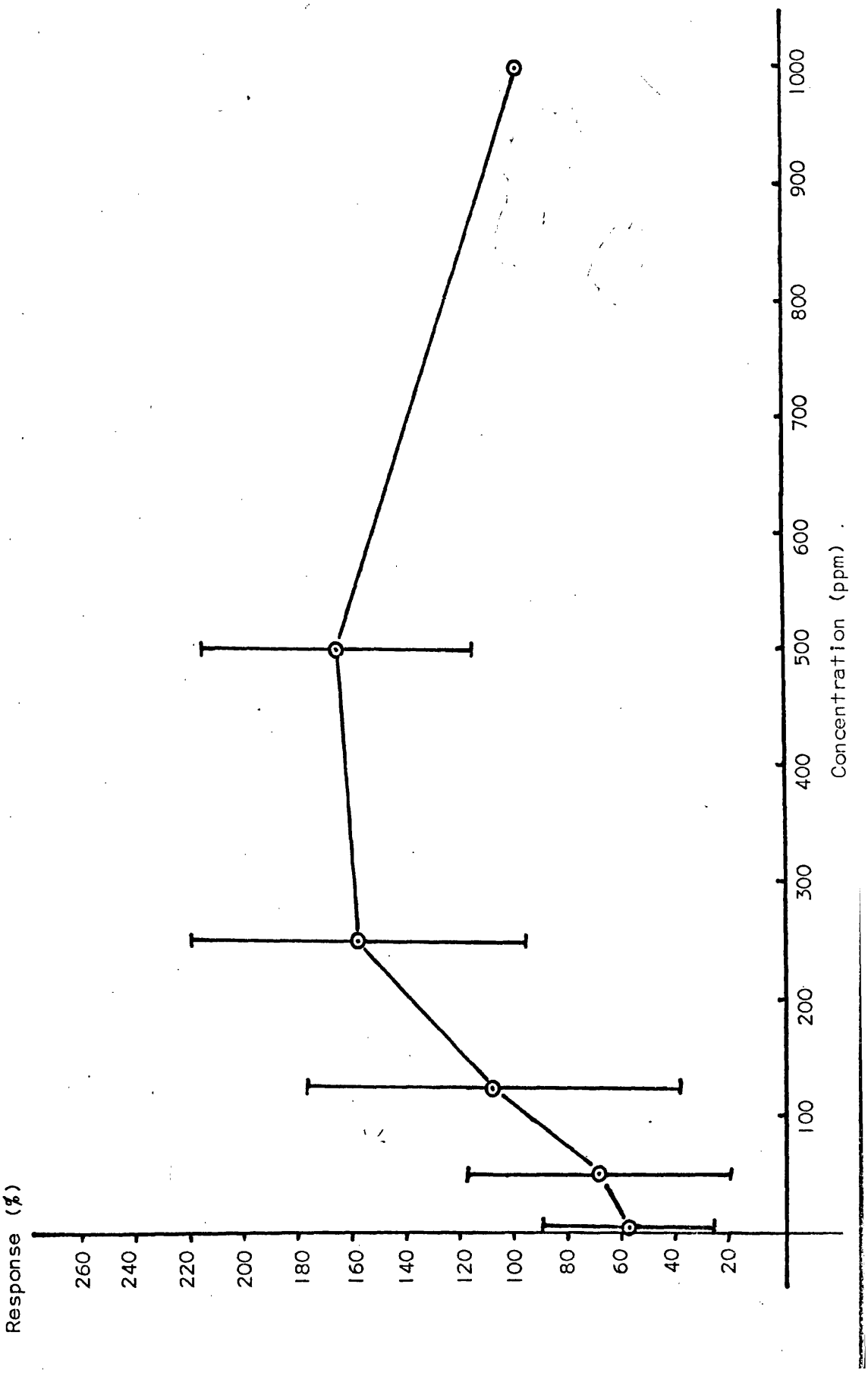
Experiments using sodium chloride solutions as epithelial stimulants were carried out during the upstream migration (18.1.71 - 29.1.71) and spawning (4.5.71 - 5.5.71) of River lampreys. 10 experiments were carried out consisting of 6 early experiments (4 males and 2 females) and 4 later experiments (2 males and 2 females). Early experiments were carried out at temperatures of 5.5 - 7.5° C and later experiments at 13.5 - 17.0° C. Stimulant concentrations of the early experiments were 1000 ppm, 250 ppm, 200 ppm, 150 ppm, and 100 ppm (Figure 29) while in later experiments 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm and 5 ppm were used (Figure 30). Figure 31 shows the mean percentage responses recorded during both the early and later experiments, and indicates that the response/concentration relationship was linear in each case up to a concentration of 250 ppm. In early experiments, mean percentage responses were not significantly different to those of the later experiments, although they were consistently less than the later ones.

Using the response to 1000 ppm solution as the 100% response, the olfactory lobe activity evoked by the other concentrations were assessed as in the 1,2-diaminoethane experiments. Mean percentage responses were plotted on a graph with the standard errors of the means (Figure 28). The relationship between concentration and response was almost linear up to 250 ppm when the responses levelled and diminished slightly at 1000 ppm. The reduction in the evoked response at concentrations above 250 ppm may have been due to inhibition caused by the relatively high pH of solutions at these concentrations. This effect was investigated later by the use of dilute hydrochloric acid as a stimulant.

Responses to stimulation with solutions of sodium chloride:

Experiments using sodium chloride solutions as epithelial stimulants were carried out during the upstream migration (18.1.71 - 29.1.71) and spawning (4.5.71 - 5.5.71) of River lampreys. 10 experiments were carried out consisting of 6 early experiments (4 males and 2 females) and 4 later experiments (2 males and 2 females). Early experiments were carried out at temperatures of 5.5 - 7.5° C and later experiments at 13.5 - 17.0° C. Stimulant concentrations of the early experiments were 1000 ppm, 250 ppm, 200 ppm, 150 ppm, and 100 ppm (Figure 29) while in later experiments 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm and 5 ppm were used (Figure 30). Figure 31 shows the mean percentage responses recorded during both the early and later experiments, and indicates that the response/concentration relationship was linear in each case up to a concentration of 250 ppm. In early experiments, mean percentage responses were not significantly different to those of the later experiments, although they were consistently less than the later ones.

FIGURE 28 Assessed integrator responses to solutions of isoleucine methyl ester expressed as percentages of the assessed integrator response to a 1000 ppm solution of isoleucine methyl ester.



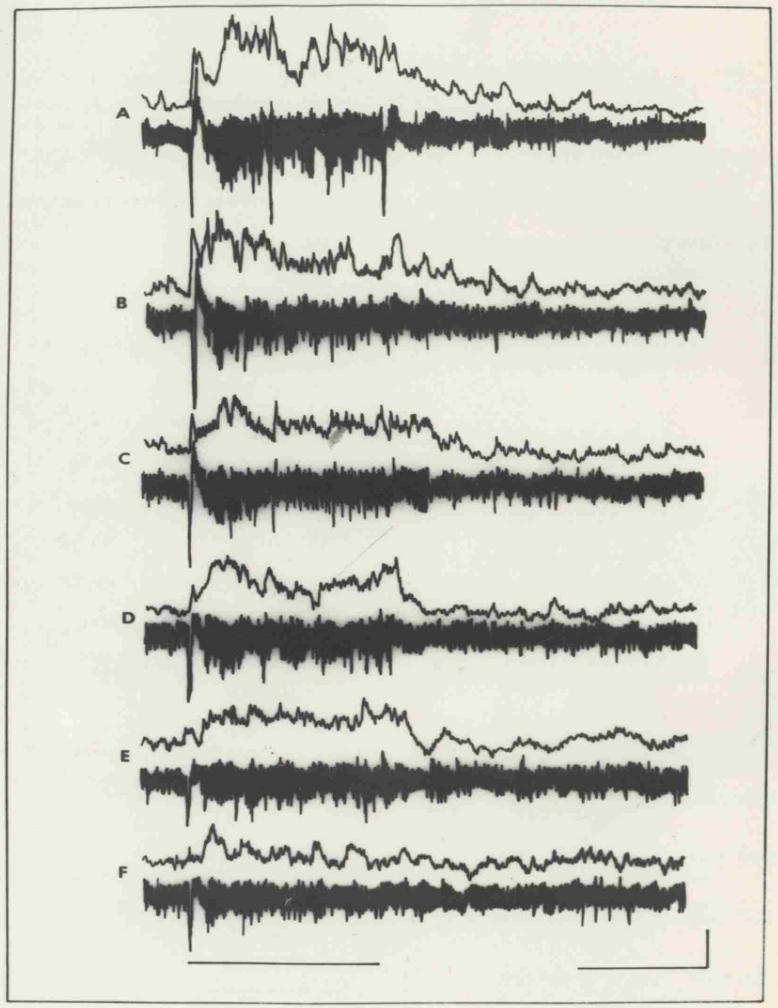


FIGURE 29

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey in response to stimulation of the olfactory epithelium with distilled water solutions of sodium chloride.

Date of experiment	29.1.71
Length of lamprey	34.6 cm
Weight of lamprey	71.8 gm
Sex	Female
Temperature of experiment	7.5° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

A 1000 ppm NaCl solution

B 300 ppm NaCl solution

C 250 ppm NaCl solution

D 200 ppm NaCl solution

E 150 ppm NaCl solution

F 100 ppm NaCl solution

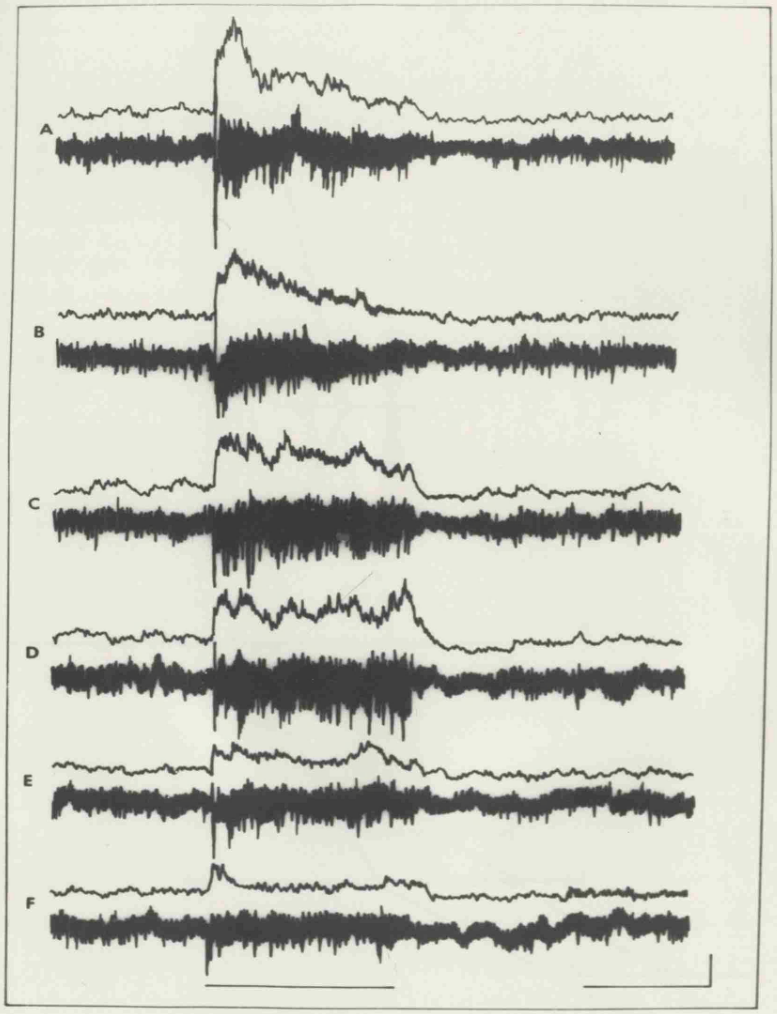


FIGURE 30

Montage of olfactory lobe activity recorded from a spawning River lamprey in response to stimulation of the olfactory epithelium with distilled water solutions of sodium chloride.

Date of experiment	5.5.71
Length of lamprey	29.7 cm
Weight of lamprey	55.9 gm
Sex	Male
Temperature of experiment	13.5° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

A 1000 ppm NaCl solution

B 750 ppm NaCl solution

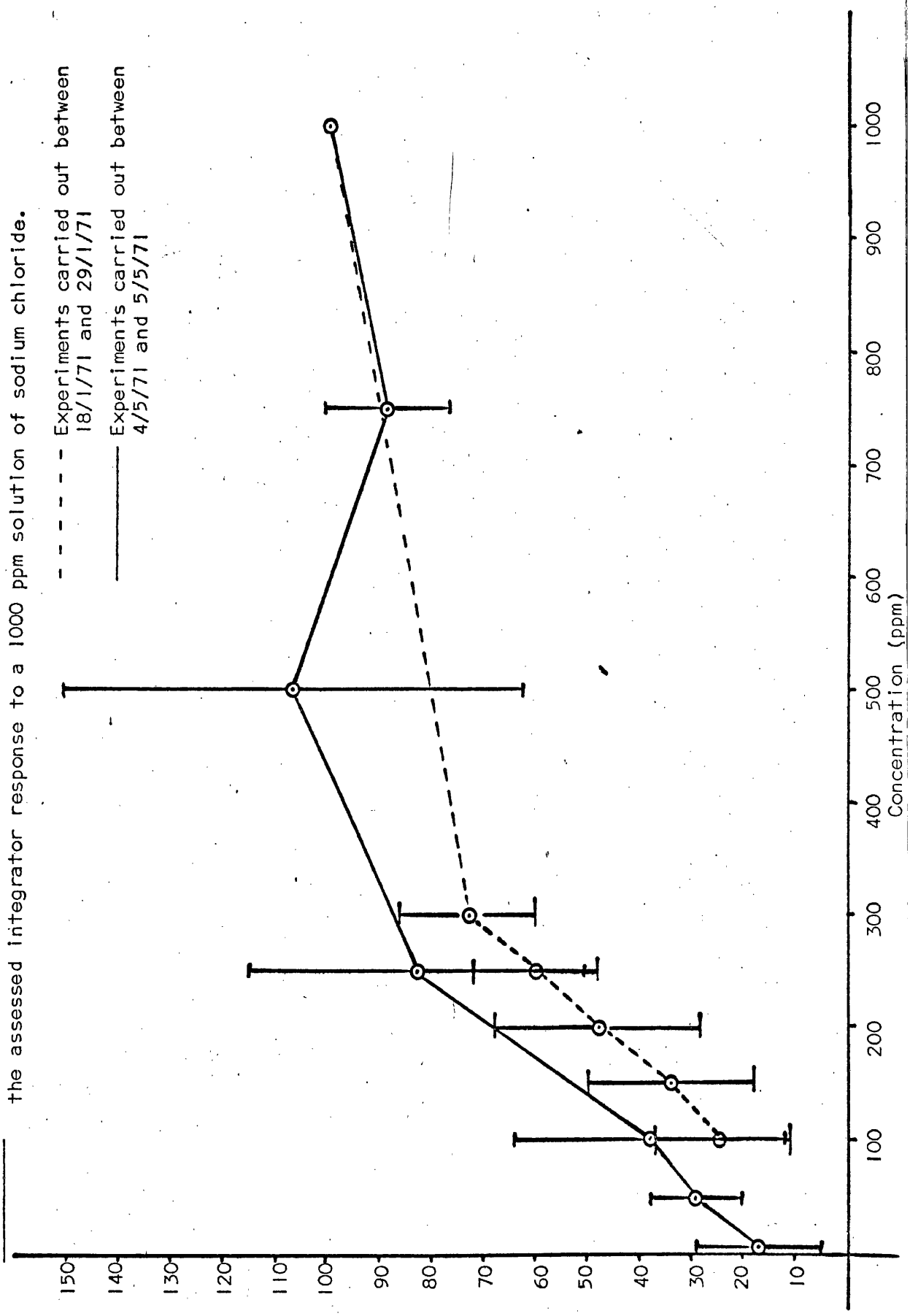
C 500 ppm NaCl solution

D 250 ppm NaCl solution

E 100 ppm NaCl solution

F 50 ppm NaCl solution

FIGURE 31 Assessed integrator responses to solutions of sodium chloride expressed as percentages of the assessed integrator response to a 1000 ppm solution of sodium chloride.



vi) Changes in Olfactory Lobe Activity After Epithelial Stimulation:

Some chemical stimulants evoked a prolonged after-response which immediately followed the termination of stimulation. Both male (Figure 32) and female (Figure 33) River lampreys exhibited the response, and although there was variation, the strength and duration of the after-response appeared to be proportional to the concentration of the stimulant solution. It became clear that the occurrence of the after-response parallelled the developing sexual maturity of the River lampreys, and the most marked after-responses were recorded during experiments carried out in late February and March.

Although the development of the after-response in anadromous River lampreys appeared to be linked to advancing sexual maturity, not all chemical stimulants evoked the response. Isoleucine (B.D.H.) failed to evoke any after-response, while isoleucine methyl ester (Koch-Light) evoked prolonged responses even at low concentrations (Figures 32 and 33). Isoleucine methyl ester was supplied as the hydrochloride $[CH_3.C_2H_5.CH.CHNH_3.COOCH_3]^+ . Cl^-$ and in distilled water, it produced an acidic solution whereas isoleucine did not (Figure 34). Dilute hydrochloric acid solutions were therefore also used as stimulant solutions in attempts to evoke after-response (Figure 35). The acid solutions frequently inhibited activity during the stimulation period, but often evoked long-term, high amplitude after-responses. From these results it was postulated that both developing sexual maturity and pH factors in stimulant solutions were related to the strength and occurrence of after-responses.

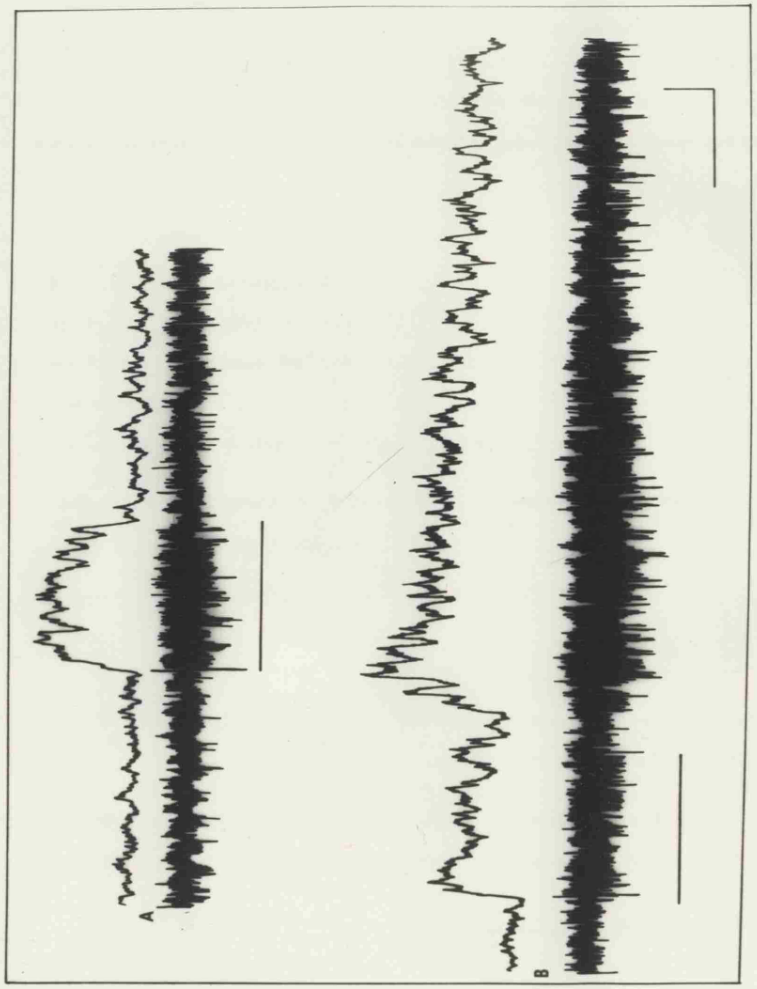


FIGURE 32

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey in response to stimulation of the olfactory epithelium with distilled water solutions of isoleucine and isoleucine methyl ester. HCl.

Date of experiment	19.3.71
Length of lamprey	29.6 cm
Weight of lamprey	48.4 gm
Sex	Male
Temperature of experiment	10.0° C
Horizontal calibration	10 sec.
Vertical calibration	50 µV

A Olfactory lobe response to epithelial stimulation with 1000 ppm distilled water solution of isoleucine

B Olfactory lobe response to epithelial stimulation with 1000 ppm distilled water solution of isoleucine methyl ester. HCl.

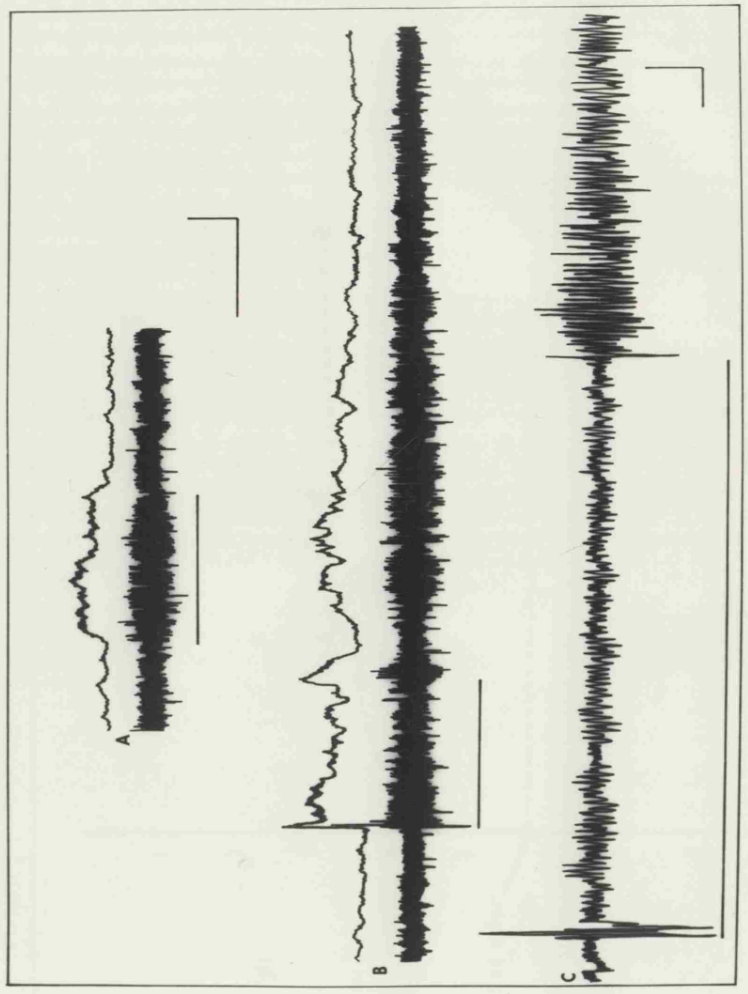


FIGURE 33

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey in response to stimulation of the olfactory epithelium with distilled water solutions of isoleucine and isoleucine methyl ester. HCl.

Date of experiment	18.3.71
Length of lamprey	31.6 cm
Weight of lamprey	59.2 gm
Sex	Female
Temperature of experiment	9.5° C
Upper two traces : Horizontal calibration	10 sec
Vertical calibration	50 µV
Lower trace : Horizontal calibration	1 sec
Vertical calibration	50 µV

A Olfactory lobe response to epithelial stimulation with 1000 ppm distilled water solution of isoleucine

B & C Olfactory lobe responses to epithelial stimulation with 1000 ppm distilled water solution of isoleucine methyl ester. HCl.

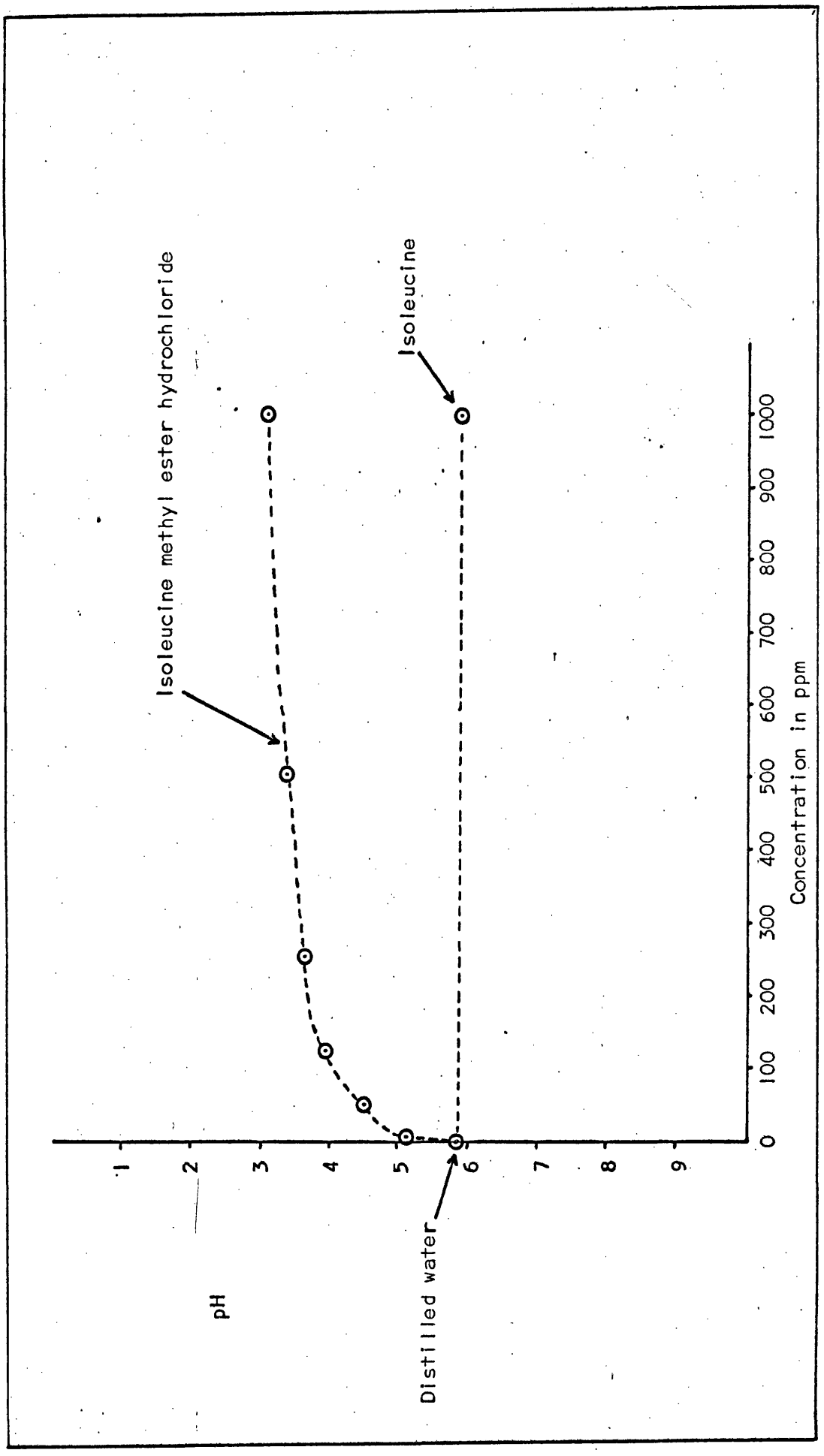


FIGURE 34 Relative pH values of distilled water solutions of Isoleucine methyl ester hydrochloride and Isoleucine at different concentrations

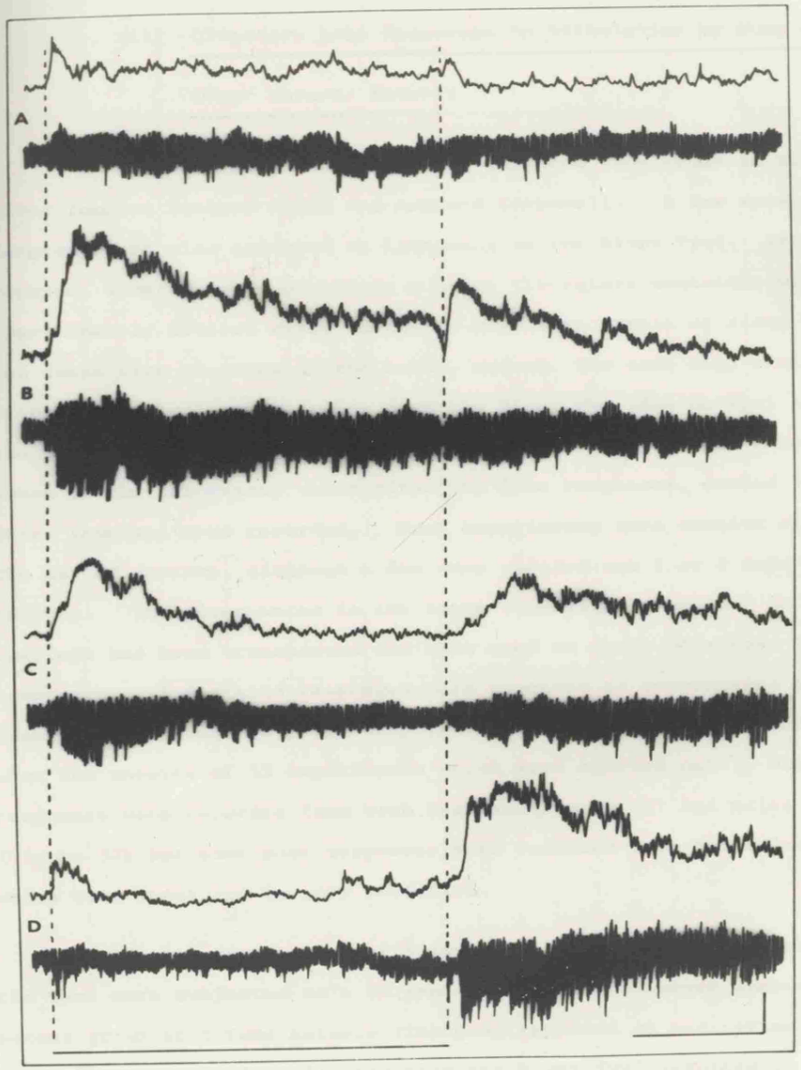


FIGURE 35

Montage of olfactory lobe activity recorded from an upstream migrant River lamprey in response to stimulation of the olfactory epithelium with dilute distilled water solutions of hydrochloric acid.

Date of experiment	12.3.71
Length of lamprey	34.7 cm
Weight of lamprey	70.0 gm
Sex	Female
Temperature of experiment	8.5° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

Four traces of responses evoked by different strengths of dilute hydrochloric acid are shown.

A 0.0001 N HCl (pH 3.90)

B 0.00025N HCl (pH 3.43)

C 0.0005 N HCl (pH 3.13)

D 0.001 N HCl (pH 2.89)

The pH of the distilled water used in this experiment was 5.49.

vii) Olfactory Lobe Responses to Stimulation by Home and Other Natural Waters:

Spawning River lampreys were captured from redds on the River Teme at Tenbury Wells and Ashford Carbonell. A few spawning lampreys were also captured at Llangadog on the River Tywi. After capture, lampreys were placed in a large fibreglass container with approximately 100ℓ of river water. A one litre sample of river water was taken from the area of the redds, and on the same day, similar river water samples were taken from the River Wye (Hay-on-Wye) and the River Usk (Usk). The lampreys and river water samples were then taken to the laboratory where olfactory lobe responses, evoked by the water samples, were recorded. Most experiments were carried out on the day of capture, although a few were carried out 1 or 2 days after capture. The river water in the large fibreglass container in which lampreys had been transported was also used to evoke olfactory lobe responses. Integrated responses were assessed as percentages of the home water response as previously described, and Tables 5, 6 and 7 show the results of 15 experiments which were carried out. Similar responses were recorded from both females (Figure 36) and males (Figure 37) but some poor responses were recorded from those lampreys which were spent and in poor condition.

In the first group of 8 experiments (Table 5) lampreys from the Teme were subjected to a 15 sec. stimulation sequence whereas a second group of 5 Teme animals (Table 6) received 40 sec. stimulations. The few spent animals collected from the River Tywi resulted in only two successful attempts to record olfactory lobe activity (Table 7), using a 40 sec. stimulation sequence.

TABLE 5

EXPERIMENTAL DATA						PERCENTAGE RESPONSES TO RIVER WATER SAMPLES			
Expt. No.	Date	Sex	Degree of Sexual Maturity	General Condition	Length of Stimulation sec.	River Teme (Tenbury Wells) "HOME"	River Wye (Hay-on-Wye)	River Usk (Usk)	River Teme water used to transport lampreys
1	30.3.71	Male	Partly spent	Good	15	100	96.9	74.5	214.0
2	30.3.71	Female	Partly spent	Good	15	100	88.8	116.6	188.2
3	31.3.71	Male	Partly spent	Good	15	100	100.7	117.9	313.6
4	31.3.71	Female	Partly spent	Fair	15	100	98.2	103.5	351.8
5	6.4.71	Male	Partly spent	Fair	15	100	109.6	137.4	267.4
6	6.4.71	Female	Fully spent	Poor	15	100	107.2	93.4	91.0
7	8.4.71	Male	Fully spent	Poor	15	100	83.4	93.2	61.6
8	9.4.71	Male	Fully spent	Poor	15	100	67.8	78.5	84.1

TABLE 6

EXPERIMENTAL DATA						PERCENTAGE RESPONSES TO RIVER WATER SAMPLES			
Expt. No.	Date	Sex	Degree of Sexual Maturity	General Condition	Length of Stimulation sec.	River Teme (Tenbury Wells) "HOME"	River Wye (Hay-on-Wye)	River Usk (Usk)	River Teme water used to transport
9	13.4.71	Male	Partly spent	Good	40	100	77.3	103.6	223.0
10	13.4.71	Female	Fully spent	Poor	40	100	70.7	119.4	156.3
11	14.4.71	Female	Fully spent	Poor	40	100	88.3	138.4	224.3
12	14.4.71	Male	Fully spent	Poor	40	100	64.2	148.9	126.5
13	15.4.71	Female	Fully spent	Poor	40	100	254.4	102.7	387.9

TABLE 7

EXPERIMENTAL DATA						PERCENTAGE RESPONSES TO RIVER WATER SAMPLES		
Expt. No.	Date	Sex	Degree of Sexual Maturity	General Condition	Length of Stimulation sec.	River Tywi (Llangadog) "HOME"	River Usk (Usk)	River Teme (Tenbury Wells)
14	23.4.71	Male	Fully spent	Poor	40	100	125.3	85.8
15	23.4.71	Female	Fully spent	Poor	40	100	82.1	72.7

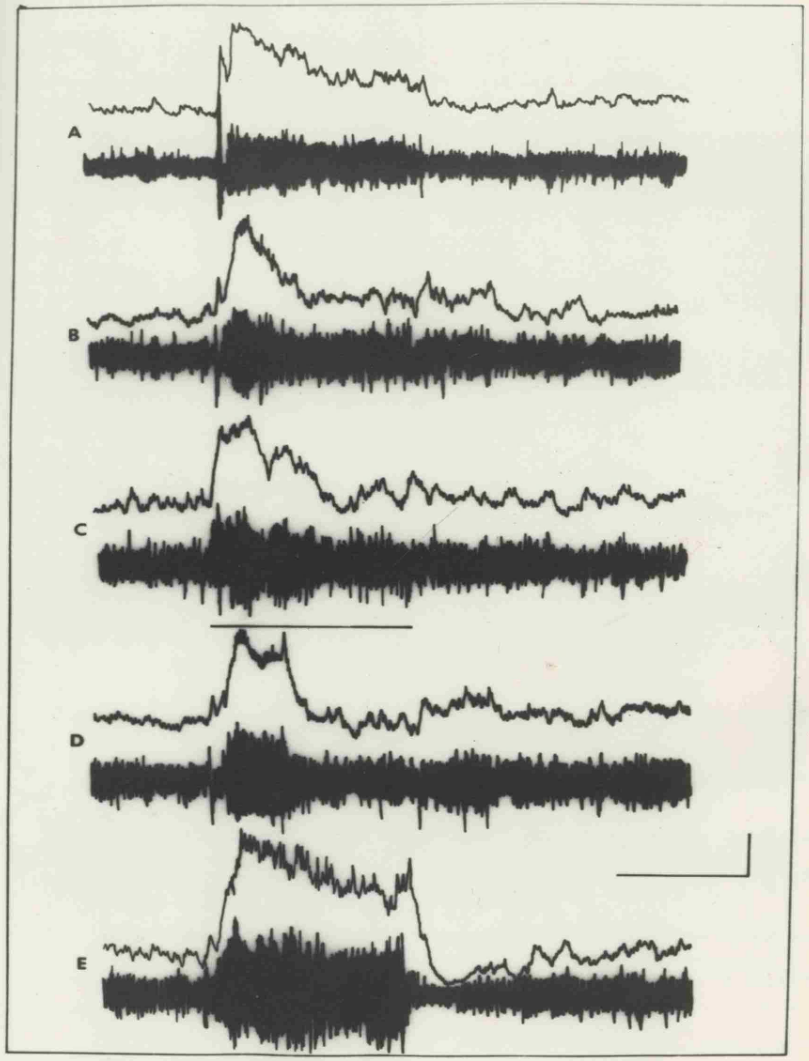


FIGURE 36

Montage of olfactory lobe activity recorded from a spawning River lamprey in response to stimulation of the olfactory epithelium with river waters.

Date of experiment	30.3.71
Length of lamprey	33.6 cm
Weight of lamprey	62.7 gm
Sex	Female
Temperature of experiment	11° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

Traces show responses to the following stimulant solutions:

A 1000 ppm distilled water solution of 1,2-diaminoethane

B River Teme water collected from River lamprey redds

C River Wye water collected from Hay-on-Wye

D River Usk water collected from Usk

E River Teme water used to transport spawning and spent River lampreys to the laboratory.

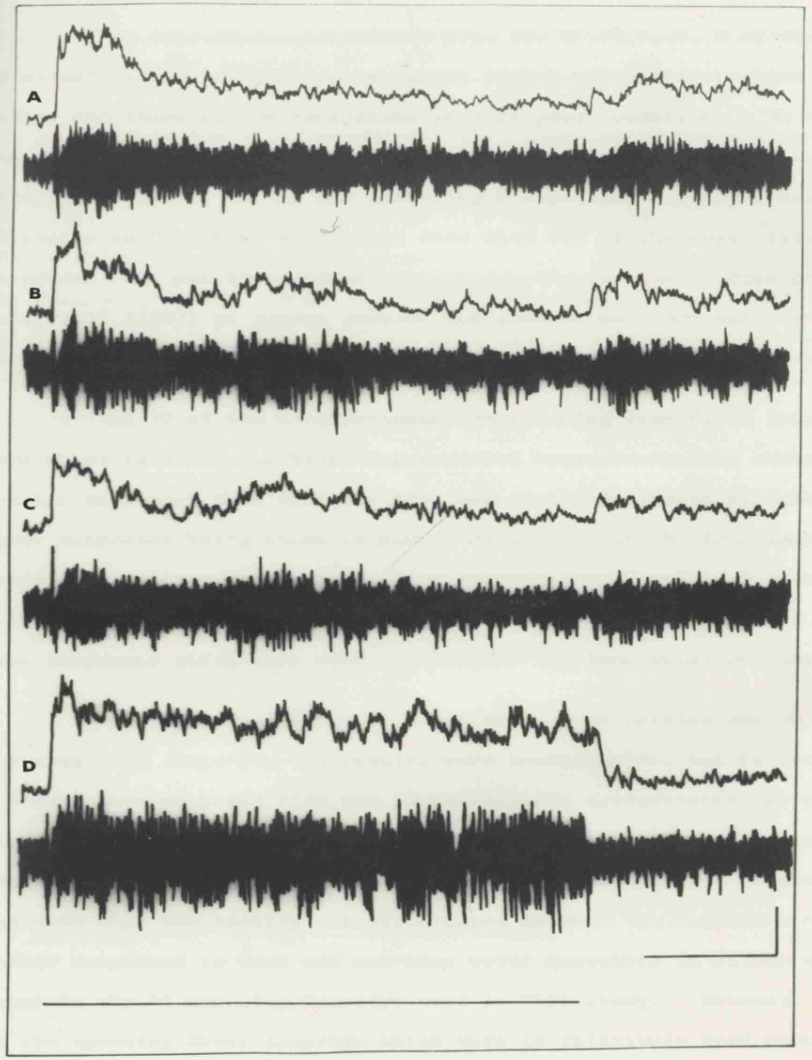


FIGURE 37

Montage of olfactory lobe activity recorded from a spawning River lamprey in response to stimulation of the olfactory epithelium with river water.

Date of experiment	13.4.71
Length of lamprey	30.3 cm
Weight of lamprey	53.2 gm
Sex	Male
Temperature of experiment	12° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

Traces show responses to the following river waters:

A River Usk water collected at Usk

B River Wye water collected at Hay-on-Wye

C River Teme water collected from River lamprey redds at Tenbury Wells

D River Teme water used to transport spawning and spent River lampreys to the laboratory.

In experiments on animals from the River Teme, 9 of the 13 animals exhibited greater responses to Usk water than to home (Teme) water, and three of the exceptions were in poor condition. In 4 of the 13 experiments, the evoked responses to Wye water were greater than home (Teme) water, and of the remaining 9 experiments, only 2 exhibited responses to Wye water which were less than 70% of the home (Teme) response. To put these results in perspective, in the studies of Ueda *et al* (1967) on homing chinook and coho salmon, average non-home water responses were never greater than 50% of the home water response.

In 10 of the 13 experiments on spawning Teme River lampreys, Teme water in which the River lampreys had been transported evoked greater responses than ordinary Teme water, the 3 animals exhibiting lower responses being those in poor condition. Of the 10 animals exhibiting greater responses to "lamprey-transport" water, 9 gave responses of more than 150% of the home water response, and 7 of these gave responses which were more than double the home water response.

As only two home water experiments were carried out on spawning Tywi lampreys, the results were inconclusive, but in total, the responses recorded from the 15 experiments demonstrated two points. Firstly, the variability of evoked olfactory lobe responses in spawning and spent River lampreys is very evident, and secondly the results indicate that the clearly cut differences between the magnitude of bulbar responses to home and non-home water described in salmon did not occur in the 15 spawning lampreys used in this study. However, most of the spawning River lampreys which were in relatively good condition did strongly respond to natural river water which had contained lampreys of the same species, and these results are particularly relevant in

the light of the electroencephalographic studies of young salmon carried out by Oshima *et al* (1969b) and discussed later.

Kleerekoper and Mogensen (1959) have shown that residence of fish in water causes it to contain a complex series of chemical components, and the loss of eggs or milt by sexually mature fish is likely to further add to this component list. Filtered solutions obtained by shaking either lamprey milt or eggs (1 gm) in distilled water (250 ml) evoked strong olfactory lobe responses when they were applied to the olfactory epithelium of spawning River lampreys (Figure 38). Filtered solutions derived from eggs and milt of *L. planeri* were also stimulating to River lampreys and some strong responses to the *planeri*/milt solutions were recorded (Figure 38). When brook lampreys were manually stripped in order to expel 1 gm of milt, a small quantity of blood was also frequently lost and this contamination of stimulant solutions by blood may account for the greater *planeri*/milt responses.

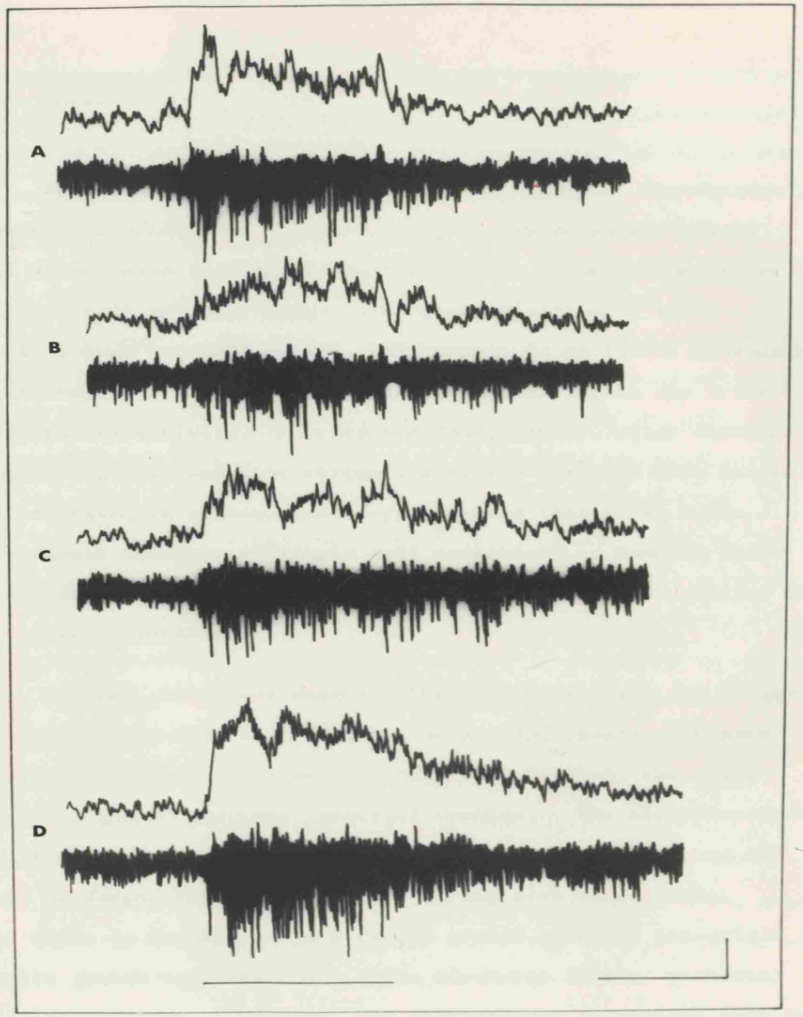


FIGURE 38

Montage of olfactory lobe activity recorded from a spawning River lamprey in response to stimulation of the olfactory epithelium with filtered suspensions of lamprey milt and eggs.

Date of experiment	10.4.71
Length of lamprey	28.4 cm
Weight of lamprey	45.7 gm
Sex	Male
Temperature of experiment	14.5° C
Horizontal calibration	10 sec
Vertical calibration	50 µV

Traces show responses to the following filtered suspensions:

A 1 gm of *fluviatilis* eggs in 250 ml distilled water

B 1 gm of *fluviatilis* milt in 250 ml distilled water

C 1 gm of *planeri* eggs in 250 ml distilled water

D 1 gm of *planeri* milt in 250 ml distilled water

D. DISCUSSION

Potential changes recorded from the brains of vertebrates were thought to arise from a summation of the spike potentials of cortical brain cells (Adrian and Mathews 1934). The rhythm of the summed potentials was attributed to a synchronisation of the "spontaneous beat" of brain cells, and Lorente de Nó (1938) postulated that "reverberating" neuronal circuits and chains within the brain synchronised the activity. Gerard and Young (1937), after working on the frog brain, presumed that rhythmic activity resulted from brain cells discharging in unison, and they suggested that gross brain activity should resemble a "single cell potential". Some of their recordings from the frog brain show potential changes very similar in form to spike potentials.

However, Libet and Gerard (1938) and Schweitzer and Wright (1938), applied nicotine to the brain surface and showed that even when synaptic connections between neurons were blocked, the brain surface still showed rhythmic potential changes. The microelectrode studies of the cerebral cortex made by Li and Jasper (1953) and the later work of Jasper (1960) showed that during deep anaesthesia, cortical cells do not discharge although graded synaptic potentials of brain cells (which regulate their spike discharge firing patterns) can still be recorded. Further, the simultaneous recordings made by Li and Jasper of cortical unit activity and surface E.E.G. do not show the correspondence postulated in earlier theories. Li and Jasper thus concluded that graded inhibitory and excitatory synaptic potentials are

the main source of E.E.G. wave patterns. However, Jasper, Ricci and Doane (1958) have stated that "the manner in which electrical waves are generated on the cortical surface is still obscure and their true functional significance is even more uncertain."

Segura and de Juan (1966) considered that "in many respects there are no qualitative differences in brain waves along the phylogenetical scale," and thus the brain activity of cyclostomes is of importance because the animals are derived from the earliest vertebrates. Gilbert *et al* (1964) studied the E.E.G. of sharks with the hope that E.E.G. analysis might "reveal features of brain function established 350 million years ago." The analysis of the brain activity of lampreys has endorsed the observations of Segura and de Juan in that qualitatively the activity does not deviate from the basic vertebrate pattern. Quantitatively the activity recorded from some parts of the lamprey brain resembles that recorded from similar areas of the brain of higher vertebrates (Table 4), and the telencephalon exhibits the greatest conformity of activity in lampreys, sharks and teleosts, which all show activity within the narrow limits of 4 - 10 Hz at 30 - 100 μV. Electrophysiological results therefore support morphological findings that in a wide range of animals, "the olfactory bulbs and centres are essentially similar in structure and form, the one relatively unchanged part of a brain which has undergone a series of complicated developments" (Allison 1953).

A common feature of mesencephalic activity evident in the E.E.G.s of lampreys, sharks and teleosts is the "arousal reaction" based on the difference between optic lobe activities in darkness and in light. In the dark, optic lobe activity is of slightly higher

frequency (5 - 14 Hz) and greater amplitude than that of the olfactory lobe, but in light the frequency of optic lobe activity increases to relatively high levels (14 - 35 Hz) at low amplitudes (usually less than 40 μV). Mesencephalic activity is variable in higher vertebrates, but mid brain arousal reactions resulting from photic and acoustic stimulation still involve increased frequencies and decreased amplitudes.

In light the optic lobes of the lamprey brain exhibit frequencies (25 - 35 Hz) and amplitudes (20 - 35 μV) which are in accordance with an arousal condition.

The activity of the medulla oblongata of fish is characterised by potential changes associated with the regulation of respiration. The frequency of medullary potentials is usually the same as that of the coincident gill or operular beat. However, although the medullary activity of lampreys resembles that of sharks (Gilbert *et al* 1964) in form and amplitude, the potentials of the teleost medulla oblongata appear to be of much lower amplitudes (Shadé and Weiler 1959). The activity of the lamprey medulla oblongata was partly composed of a high-frequency, low amplitude component which was difficult to assess because of the vigorous respiratory potentials. However, this element may be comparable to the high frequency (14 - 32 Hz) low amplitude activity recorded by Enger (1957) from the medulla oblongata of the cod brain. Gerard and Young (1937) recorded spike-like potentials from the medulla of the frog brain, and it is probable that the spike-like activity characteristic of the medullary activity of lower vertebrates, controls the respiration rate by regulation of the discharge of efferent nerves to the respiratory musculature.

Some recordings of activity from the medulla oblongata of the lamprey brain include potential disturbances which have the same frequency as the coincident heart beat (see Figure 16). The high amplitude medullary activity which can be recorded from both the lamprey and the shark brain indicates that the medullary somatic motor elements of these brains are dominant.

It was not possible, because of the high amplitude medullary potentials linked with respiration, to record any medullary responses to photic stimulation. However, optic lobe responses were recorded when light was flashed onto the lateral eye of the lamprey. The response consisted of positive potentials at the on- and off- of a 200 msec. flash, followed by a phase of negative activity. The first of the four responses shown in Figure 16 is characteristic of the pattern of response. Both Buser (1950) and Schadé and Weiler (1959) after recording from the brains of teleosts, postulated that the phases of optic lobe responses were derived from (i) the potential evoked at the synaptic connection of optic nerve fibres and tectal neurons, and (ii) the activity subsequently evoked in deeper mesencephalic cell layers.

The distinct on- and off-responses recorded from the optic tectum of the lamprey were attributed to "on" and "off" elements in the retina by Veselnikin (1966), who in an earlier study of *L. fluviatilis* (1963) found that the optic lobe response to short light flashes was variable, but mainly consisted of one or more positive spikes followed by a negative phase of activity. Repeated flashes were found to cause responses which were of diminished

amplitudes. A similar effect is shown in the responses to four flashes of light shown in Figure 16. Veselnikin (1963) postulated that the diminished responses resulted from fast exhaustion in the lamprey nervous system, and the effect, which has yet to be investigated, was compared to the diminished responses evoked by rhythmic stimulation and recorded from the occipital cortex of the new-born child, (Ellingson, 1960).

During this study, no responses to photic stimulation were evident in olfactory lobe activity and a similar absence of telencephalic response to photic stimulation was reported by Veselnikin (1966). Kleerekoper (1971) has concluded that this demonstrates the hypothesis of Karamian *et al* (1966) that the visual neural pathway in lampreys is exclusively retino tectal.

Responses were evoked in the optic lobe of the ammocoete brain by photic stimulation of the head and tail regions (Figure 14). Veselnikin (1963) showed that photic stimulation of the pineal complex evokes responses in the optic tectum of adult *fluviatilis*, and thus, the ammocoete with its non-functional (Kleerekoper 1972) lateral eyes can still respond to photic stimulation of the head. However, although the tail region of the ammocoete has been shown to be photosensitive (Young, 1935a; b; Francis and Horton, 1936; Steven, 1950; 1963; Harden-Jones, 1955), the neural pathway for information from dermal receptors is thought to be the lateral line nerve rather than the spinal cord (Young 1935a). In addition, the behavioural reaction time for dermal stimulation is long (Steven 1950, 1963). This suggests that the optic lobe responses to photic stimulation of the

ammocoete tail may arise in different receptors from those whose behavioural effects were described by Young (1935a, b) and Stevens (1950, 1963). The mechanism of dermal photosensitivity in lampreys requires more electrophysiological study especially since both skin cells (Stevens 1950, 1963) and some nerve cells (Young 1935a) may be photosensitive.

An interesting feature of the electrical activity of the ammocoete brain is the apparent dominance by the activity of the olfactory lobe (Figure 14). The olfactory end-organ of the ammocoete is little more than a ciliated pit (Kleerekoper and Van Erkel 1960), whose function in the larval stage is not known. Although Kleerekoper (1972) doubts the existence of an olfactory sense in the pre-metamorphic lampreys, the electrical activity of the ammocoete olfactory lobe recorded in this study, indicates that at least the telencephalic component of the olfactory system is operational. The functioning and level of discrimination of the ammocoete olfactory system requires further study, but electrophysiological results indicate that in the ammocoete, the olfactory lobe is already the electrically dominant brain structure.

The electrical activity of the brain of a newly transformed *L. fluviatilis* closely resembles that of the adult (Figures 15 and 16). However, the medullary activity of the newly transformed animal resembles that of the ammocoete medulla in that spike potentials occur in short bursts rather than in a rhythmic sequence as in the adult. The small size of ammocoetes and newly transformed animals led to difficulties with the respiratory perfusion of their gills during

experiments, and this may have influenced recordings of their medullary activity.

In adult, upstream migrant *L. fluviatilis*, a supply of respiratory water to the gills was necessary to maintain intrinsic olfactory lobe activity. Figure 20 shows the effect of stopping gill perfusion for 45 min., on olfactory lobe activity, and the reappearance of normal activity following the resumption of perfusion. During the 45 min. period without perfusion, olfactory lobe activity diminished to a low level, while recommenced perfusion resulted in an almost immediate increase in activity so that within 5 min. activity had returned to the original level. These results parallel the effects of terminating gill perfusion on medullary activity and optic lobe responses in *L. fluviatilis* as described by Veselnikin (1963). Medullary activity gradually diminished, but reappeared 15 - 20 sec. after resumption of gill perfusion.

In the later stages of the 45 min. period without gill perfusion, only faint and irregular heart beats could be recorded, and the reduction of olfactory lobe activity presumably resulted from the reduced supply of oxygen to the neurons of the olfactory lobe.

The work of Dupé (1968) on the effects of increased temperature on the frequency of olfactory lobe activity in a lungfish *(Protopterus)*, showed that if the fish were warmed or cooled over a 2-day period, the Q_{10} (20-30°C) relationship between frequency and temperature was variable, with values up to 1.91. Work on hypothermic rabbits carried out by Putknonen and Sarajas (1968) showed a Q_{10} (25 - 35°C) relationship of 2.3 between the frequency of olfactory lobe

activity and temperature. In that study the rabbits were effectively made poikilothermic in that hypothermia was achieved by minimising their thermoregulation with anaesthetics before cooling.

These studies of poikilothermic subjects showed that in the short-term they exhibited little acclimation, and the normally assumed Q_{10} effect, i.e. an approximate doubling of the rates of physiological or metabolic activities for each $10^{o}C$ rise in temperature, was evident. In this context, Bullock (1935) has stated that many cold-blooded animals are "relatively independent of the temperature within limits in nature. That is to say that these species tend to maintain a certain level of metabolism and other characters, measured as rates, compensating for different temperatures by homeostatic mechanisms of various kinds." Hensel and Hildebrandt (1964) regarded poikilotherms of this type as "adjusters" in that their metabolic rate is stabilised against changes in their body temperature, and cited as an example the medusa *Aurelia aurita* because its rate of swimming movements was approximately the same at habitat temperature of $14^{o}C$ and $29^{o}C$.

During the upstream migration of adult *fluviatilis* (October-March), the animals are exposed to an ambient temperature range of $3-15^{o}C$, and an attempt was therefore made to assess any relationship between temperature and the frequency of olfactory lobe activity during this period. Lampreys were maintained in a concrete holding pond and experiments were carried out throughout the period, at the temperature of the pond water on the day of the experiment. The results from 37 experiments are shown in Figure 18. The linear

relationship between temperature and frequency resulted in a Q_{10} (0-10°C) of 1.26, with frequencies of 5.7 Hz at 0°C and 8.5 Hz at 17.5°C. It is therefore postulated that upstream migrant River lampreys are partly able to acclimate to changing environmental temperatures over the autumn/spring period of migration. The compensatory mechanisms responsible for acclimation require further physiological and biochemical study because the control of brain activity, under changing environmental conditions must be important in the physiology of migratory fish involved in complex behavioural patterns.

The initial response recorded from the olfactory lobe during stimulation of the olfactory epithelium consisted of a diphasic potential lasting approximately 300 msec. and of relatively high amplitudes (200-300 μV) (Figure 21). These potential changes probably represent a series of potentials which have been recorded (Bruckmoser, 1971) from different areas and depths within the olfactory lobe. Bruckmoser (1971) used microelectrodes to show that three components were apparent in the response of the lobe to electrical stimulation of the olfactory nerve. These components represented both the presynaptic potential of the olfactory nerve fibres and the neuron potentials subsequently evoked from secondary and tertiary neurons. Recordings of gross brain activity displayed on a pen recorder and relayed by Ag/AgCl macroelectrodes, have insufficient definition to show these potentials in the detail recorded by Bruckmoser (1971).

However, the synchronised high amplitude waves recorded after this phase and throughout short-term (15-40 sec) stimulation

were similar to those recorded from the olfactory bulb of the rabbit by Adrian (1950c) who considered that the waves resulted from the summated activity of mitral cell dendrites. Ottoson (1959) has shown that antidromic stimulation of the olfactory bulb of the frog during olfactory stimulation of the epithelium, stops the induced waves, and he concluded that the synchronised activity is post-synaptic. Ottoson (1959) further considered that the regular waves were derived from the synchronous activity of secondary olfactory neurons, and cited as evidence, Adrian's (1950a) recordings from centrally directed olfactory neurons, of groups of impulses associated with each surface wave.

Recordings of evoked responses in the olfactory lobe of the River lamprey showed variations in both the magnitude and pattern of activity, which were dependent on the stimulant. Thus, 3000 ppm NaCl evoked a much greater response than 3000 ppm morpholine, while the effect of 3000 ppm isoleucine methyl ester markedly differed from both, in that it evoked a response which consisted almost exclusively of peaks of activity which occurred when the stimulant was turned on and off. Responses to NaCl and morpholine were composed of high initial activity which gradually decreased during the period of stimulation. Although these responses were not further analysed in this context, they indicated that in the River lamprey a degree of discrimination was present in the olfactory lobe responses to different stimulants. Studies on other animals have investigated this aspect more thoroughly, and Adrian (1950a, b, 1953) showed that responses recorded from within the olfactory bulb of mammals, varied in the latency, duration, rate of growth and rate of decay of spike activity

when evoked by different stimulants. A frequency analysis of the olfactory bulb responses to several odours in rhesus monkeys (Hughes and Mazurowski, 1962) showed that each odour evoked a specific pattern of changing activity frequencies during the period of stimulation. Although further investigation is necessary, the preliminary study of qualitative differences in the olfactory lobe responses of the River lamprey indicate that in this animal, as in others, some olfactory discrimination takes place in the olfactory lobe of the brain.

Olfactory lobe responses were studied as a function of stimulus concentration by using three chemicals (1,2-diaminoethane, isoleucine methyl ester and sodium chloride) at several concentrations. The results (Figures 25, 28 and 31) show the same general shape of concentration/response curve for all three chemicals in that in each case the response increased as a decelerated function of concentration. Thus in all three graphs a rapid initial increase in the magnitude of response is evident, especially in the case of isoleucine methyl ester where a 5ppm solution evoked approximately one third of the maximum response recorded at a concentration of 500 ppm. This high degree of olfactory sensitivity to low concentrations of isoleucine methyl/ester may be common to predatory lampreys, because the behavioural studies of Kleerekoper and Mogensen (1963) showed that this compound was an attractant in the feeding behaviour of parasitic Sea lampreys and elicited strong locomotor responses at a concentration of 0.2 ppm. The anadromous River lamprey is also responsive to low concentrations of the compound which may be a common olfactory attractant in fish predator/prey behaviour (Kleerekoper 1963).

Some attempts at quantitative olfactory lobe responses were made by Dupé and Godet (1969) who recorded from the dipnoid fish *Protopterus annectans* and showed that increased stimulus concentration led to increased amplitude and duration of olfactory lobe responses. Hara and Gorbman (1967) used NaCl solutions to evoke olfactory bulb responses in the goldfish and recorded that "the response to NaCl increased in magnitude linearly with increasing concentration within the range tested" (600-3000 ppm). Figure 31 shows that the olfactory lobe responses of the River lamprey to concentrations in this range have in fact reached a maximum and in some cases have even diminished at the highest concentration indicating perhaps that the olfactory system of the River lamprey may be more sensitive than that of the goldfish to NaCl in solution.

Mozell (1958) also recorded diminished responses at high concentrations in his work on unit activity recorded from the olfactory bulb of the rabbit during stimulation of the olfactory epithelium with amyl acetate, heptane, benzene and ether. Even though the concentration response curves described by Mozell (1958) were recorded by a different technique and from a mammal, Mozell (1958) noted that "discharge strength and duration increased approximately as a negatively accelerated function of concentration". The close similarity in the responses from the olfactory lobes of two widely separated animals perhaps further indicates the unchanging nature of olfactory brain activity throughout vertebrates, although Mozell gave no explanation for the "downward turn in the stimulus-response curve at higher concentrations". The work of Hara and Gorbman (1967) on the olfactory system of the goldfish may be relevant in this context,

because they found that the gross activity recorded from the olfactory bulb during chemical stimulation of the olfactory epithelium on one side of the fish, was strongly inhibited by simultaneous electrical stimulation of either the contralateral bulb, the anterior commissure or the posterior regions of the homolateral telencephalon. When olfactory bulb responses during NaCl stimulation were investigated by microelectrode studies of unit activity (Hara 1967a), 6 different unit response patterns were described which included some cells that were either inhibited or facilitated during stimulation. Electrical stimulation of the contralateral bulb during chemical stimulation resulted in some inhibition of the activity of all the cell types.

Hara and Gorbman (1967) considered that these results indicated that the control of olfactory bulb activity in the goldfish was determined by both the activity of the contralateral bulb through the anterior commissure and by the activity of more posterior regions of the brain. That central regulation of olfactory bulb activity can occur is now accepted on this and other anatomical and electrophysiological evidence. Thus, Cajal (1911) and Allison (1953) showed that the olfactory bulb receives fibres from both the basal telencephalic regions and the contralateral bulb, while Kerr and Hagbarth (1955) and Callens and Boisacq-Schepens (1963), working on mammals, and Døving and Gemne (1966) and Døving (1966) working on the burbot, have all demonstrated electrophysiologically that efferent stimulation both from more posterior brain regions and from the contralateral bulb (via the commissure) can affect the synchronised-activity responses of the olfactory bulb. Further evidence (Kerr and Hagbarth, 1955; Yamamoto and Iwama, 1960; Harada and Takagi, 1961;

Takagi, 1962), indicates that regulation of olfactory bulb activity is based on both inhibitory and facilitatory control systems.

It is therefore postulated that the decline in the olfactory lobe responses of the River lamprey at high stimulant concentrations occurs as a result of inhibitory control either by the action of some cells of the contralateral bulb or by other brain structures. The mechanism can only be investigated by microelectrode studies of unit activity but some of the results of Kerr and Hagbarth (1955) may be particularly relevant because they found that synchronised responses in the olfactory bulb of the cat were differently affected by changing the frequency of simultaneous electrical stimulation of the anterior commissure. Thus high frequency commissural stimulation abolished synchronous olfactory lobe activity, while low frequency stimulation enhanced it. In the cat, the transition from facilitation to inhibition occurred in the frequency range, 30-60 Hz. The fact that the River lamprey has a well-developed interbulbar commissure (Schilling, 1907) as well as other, more posterior commissures which carry olfactory fibres, may be relevant to the contralateral inhibition hypothesis, and would enable microelectrode studies to be carried out.

The after-response recorded following the termination of olfactory stimulation from the olfactory lobes of sexually maturing River lampreys is not easily explained. The characteristics of the after-response were i) that it was an increasingly apparent component in olfactory lobe activity of lampreys nearing sexual maturity, ii) that its magnitude appeared to be proportional to the stimulus strength, and iii) that it was most evident following stimulation with HCl solutions of low pH values. The acid solutions, when used to

evoke after-responses, also inhibited olfactory lobe activity during the period of stimulation, the degree of inhibition appearing to be proportional to the stimulus strength. Thus, the strongest acid solution almost entirely inhibited activity during stimulation, but evoked the strongest after-response (Figure 35).

Histochemical studies of steroidogenesis in the gonads of sexually maturing River lampreys (Hardisty and Barnes, 1968; Barnes, 1970) have shown that the period of the most intense steroid biosynthesis occurs in late February and March, a period similar to that in which maximum olfactory lobe after-responses occurred. That nasal and genital functions are linked has already been noted (Introduction to the Electrophysiological studies), and it is therefore postulated that the after-response in sexually maturing River lampreys is perhaps related to the effects of increasing levels of sex hormones. The study by Hara (1967b) of the effects of administered sex hormones on the olfactory lobe activity of the goldfish, is important because it was shown that when testosterone was administered to male fish, and estradiol to females, olfactory lobe responses to NaCl stimulation were increased, and a strong after-response occurred following the termination of olfactory stimulation in estradiol-treated females, and some naturally-maturing control females. Although this response parallels that found in sexually-maturing female River lampreys, Hara could not demonstrate after-responses in male fish, and because the magnitude of the after-response was apparently independent of the stimulus concentration, he postulated that the after-response was the result of some "sex-linked mechanical factor". However, he also noted that the response was abolished by transecting the olfactory tract,

and was therefore probably controlled by the activity of other brain regions.

The proportional relationship between after-response and stimulus strength described in both male and female River lampreys indicates that in this animal, the effect is probably not due to mechanoreceptors present only in females as postulated in goldfish by Hara, but possibly a facilitation of some olfactory lobe neurons by other brain structures which are in turn under hormonal control. Hara's observation that in his experiments the range of stimulant concentration used could have evoked "massive overstimulation" may be relevant in that a maximum level of after-response could have been reached, and low stimulus concentrations might have evoked after-responses which were proportional to stimulus concentration.

In a study of the response patterns of olfactory bulb neurons, Hara (1967a) described some cells whose spontaneous activity was completely abolished by contralateral olfactory tract stimulation, but was facilitated for some seconds after the termination of the contralateral stimulation. It is therefore possible, that after-responses in olfactory lobe activity could result from central control of some specific olfactory lobe neurons, but it is suggested that the after-responses recorded from River lampreys were also related to both chemical and hormonal factors.

The olfactory lobe responses of spawning River lampreys to stimulation of the olfactory epithelium with "home" (spawning ground) river water and water from other rivers were primarily characterised by their variability. Although Sutterlin and Sutterlin (1971) have

noted an inherent variability in the olfactory lobe responses of young salmon, the variations encountered in the responses of spawning (i.e. partly spent or fully spent) River lampreys were thought to result from the poor physiological condition of lampreys at this stage when the spawning animals are within a few days of death.

Despite their variability, the River lamprey responses to "home" water were not significantly greater than the responses to other river waters which were tested. The same comparison, when applied to the responses of salmon which were known to home, showed that "home" river water consistently evoked significantly greater olfactory bulb responses than other river water samples (Hara *et al*, 1965; Ueda *et al*, 1967; Oshima *et al*, 1969a, b; Hara, 1970).

However, the results from salmon could not, and were not intended to prove that homing actually occurred, but were investigated as part of the mechanism by which homing was achieved. Many mechanisms have been postulated for the homing behaviour of anadromous salmonids, and although several may be involved, the currently accepted theory of salmonid homing within river systems is based on an olfactory mechanism. The olfactory lobe responses of River lampreys to river waters does not therefore show whether the animal can home, but rather indicates that if homing does occur it may be accomplished by a non-olfactory mechanism.

Alabaster (1970) has shown that the upstream migration of salmon is related to downstream river flow, and elvers migrating through an estuary from the sea have been shown to be attracted by "inland" water (Creutzberg 1959). Perhaps, therefore, lampreys

entering the Bristol Channel and Severn Estuary do not "home" to particular rivers which flow into the area but are attracted to currents of fresh water which provide directional cues in complex estuarine conditions. Under these circumstances, the greatest numbers of migrants would be expected to enter the river which exerts the most effect on the estuary. Observations over three years have indicated that the largest upstream migration of River lampreys from the Bristol Channel probably takes place into the Severn and its estuary. The Severn is the largest river of the area, and in this context, Applegate and Smith (1951) showed that the migration of land-locked Sea lampreys from a lake was most intense into the largest rivers. The migration of River lampreys from the sea into a river is possibly governed therefore by the effects of the river within the inshore marine environment.

The directional movements of lampreys within river systems have not been studied, and this aspect of their migratory behaviour requires some attention. Studies of the fresh water migratory routes of fish have usually involved large-scale and costly tagging experiments, and are normally only carried out on economically important species. However, the use of subcutaneous sonic tags which have recently been developed would make it possible to track individual migrants, and thus provide immediate results for relatively little expenditure. This new development in fisheries technology could be profitably applied in the study of lamprey migration routes.

Although the olfactory lobe responses of spawning River lampreys to "home" water were no greater than responses to other river waters, "home" river water which had contained River lampreys evoked

some very large responses. White (1934a,b) suggested that the standing fish population in a river conditions the water in such a way that related returning migrants are attracted to the stream, and this suggestion is rapidly assuming credence in view of recent behavioural and electrophysiological evidence. Thus, Oshima *et al* (1969a) showed electrophysiologically that previously non-stimulant water became highly stimulant to young salmon after it had contained fish of the same species, and Nordeng (1971), on the basis of a tagging experiment with migratory and non-migratory stock of char, postulated that returning migrants were attracted to a particular river system by an attractant released by their relatives in that river. White (1934b) suggested that milt shed during spawning was a possible attractant for upstream migrant fish, and although Thunberg (1971) has shown that White's suggestion does not hold for the Alewife, olfactory lobe responses of River lampreys indicate that the discharge of milt and eggs of this species into the water, imparts a strong smell to the water to which other River lampreys olfactorily respond. Nordeng (1971) suggested that substances in the mucus were the chemical attractants for homing char.

Fontaine (1938) recorded that sexually mature sea lampreys were used by fishermen as "bait" in traps set to catch upstream migrant sea lampreys, and Morris (1957) has shown that "glandular" cells develop in the gills of sexually mature male River lampreys, which may secrete pheromone-like substances. The lamprey olfactory lobe responses to water which had previously contained lampreys showed that the presence of sexually mature animals somehow altered river water so that it became more stimulant to lampreys of the same species.

In addition, field observations indicated that lampreys of a closely related species, i.e. *L. planeri*, were attracted to the redds of *L. fluviatilis*, even though river conditions in some cases were apparently unsuitable for successful spawning by the smaller species. It therefore appears that in the River lamprey, as in some other fish species, the anadromous migratory behaviour, culminating in the aggregation of spawning individuals results at least in part from attractant substances released into the water by the sexually mature animals themselves. In the case of the River lamprey, the attractant may even be strong enough to attract animals of a closely related species for which river conditions do not permit spawning.

The behavioural effects of such substances, and their identity has not been investigated but a fruitful line of research might involve a comparison of the chemical analyses of water which had contained either immature of sexually mature lampreys. Kleerekoper and Mogensen (1959) have already analysed the chemical composition of the scent of trout in water, and a similar study of the scents of immature and sexually mature lampreys might show chemicals which occurred only in the scent of sexually mature animals and which could be isolated and used in both behavioural and electrophysiological studies of lampreys. This electrophysiological study has already shown that the stimulant action of river water is increased if sexually mature lampreys have resided in it, and chemical analysis of both lamprey mucus and the "glandular" gill cells described by Morris (1957) might indicate the source of any lamprey pheromones.

IV

CONCLUSIONS

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1. In British rivers the duration of the larval period of *L. fluviatilis* is estimated as four and a half years.

2. Transformed, downstream migrant River lampreys were captured in March and April and could not be collected from the upper reaches of rivers after this period. Downstream migration may be correlated with river flooding.

3. The downstream migrant population of the Severn is composed of at least two different size groups which may result from sexual dismorphism or variations in larval feeding conditions.

4. In the spring, a high proportion of recently caught, transformed River lampreys can be successfully transferred directly from freshwater to full-strength sea water, indicating that in the adult feeding phase, the distribution of River lampreys is not limited by salinity.

5. Lampreys which died during direct transfer exhibited abdominal swelling, resulting from an accumulation of water in the gut.

6. Although transformed lampreys did not feed in marine aquaria, they exhibited behavioural patterns similar to some of those described for feeding, landlocked Sea lampreys.

7. A correlation of autumnal river flooding with the estuarine movement of upstream migrant River lampreys indicates that strong freshwater flows provide directional cues to anadromous migrants.

8. The spring spawning of River lampreys occurs during a period of low river flow associated with increasing water temperatures, and the phases of spawning are related to river temperature.

9. Spawning River lampreys from the Teme are bigger than those from the Tywi, where spawning occurs 1 - 3 weeks later.

10. Spawning River lampreys are frequently accompanied by spawning brook lampreys *(L. planeri)* and the assembly of spawning groups may result from interspecific olfactory attractants released by sexually mature animals. Communal spawning may result in accidental hybridisation.

11. The brain activity of lampreys resembles that recorded from the brains of other lower vertebrates.

12. In the larval stage, the olfactory lobe is the electrically dominant brain structure. Optic lobe responses to photic stimulation may result from both pineal and dermal photosensitivity.

13. The electrical brain activity of newly transformed lampreys shows similarities with the brain activities of both ammocoete and adult lampreys.

14. The brain activity of upstream migrant River lampreys is dominated by high amplitude medullary potentials. Optic lobe responses to photic stimulation of the lateral eyes resemble similarly-evoked responses from the same region of the teleost brain. Gill perfusion was necessary to maintain telencephalic activity in which no responses to photic stimulation of the eyes were recorded. Dominant frequencies of olfactory lobe activity recorded during the period of upstream migration indicated that some acclimation occurred in response to increasing water temperatures.

15. Quantitative differences in the olfactory lobe responses of adult River lampreys indicate that some olfactory discrimination occurs in this part of the olfactory system.

16. Olfactory lobe responses increased as a decelerated function of concentration during quantitative tests with chemical stimulants. The decline in response at high stimulant concentrations may be due to inhibitory control by either the contra-lateral bulb or other brain structures. The River lamprey showed high olfactory sensitivity to one chemical, (isoleucine methyl-ester), which is a specific attractant in the feeding behaviour of parasitic Sea lampreys.

17. After-responses which occur following the termination of stimulation may result from a central control of some olfactory lobe neurons, the effect is also probably related to hormonal and chemical factors.

18. In contrast to the results of similar work on homing salmon, the olfactory lobe responses of River lampreys to "home" river water were not significantly greater than the responses to other river waters. This indicates that homing, if it occurs in lampreys, is achieved by a non-olfactory mechanism. River water which had contained sexually mature lampreys evoked some large olfactory lobe responses, and it is therefore postulated that sexually mature animals release interspecific attractants into river water which result in the accumulation of spawning groups.

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Communal spawning of brook and river lampreys, *Lampetra planeri* Bloch and *Lampetra fluviatilis* L.

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A description is given of the spawning of brook lampreys *Lampetra planeri* Bloch and unusually small river lampreys *Lampetra fluviatilis* L. in the same redd. No successful interspecific copulation was observed, although the possibility of accidental cross fertilization cannot be excluded.

The relationship and specific status of the European brook and river lampreys, *Lampetra planeri* and *L. fluviatilis*, has long been a subject for discussion (Léger, 1924; Weissenberg, 1925; Enequist, 1937; Young, 1962). Although hybridization of these two forms may be readily achieved by artificial fertilization, hybrids apparently have not been followed through to metamorphosis. Natural hybridization has usually been discounted on the grounds that the normal size differential between brook and river lampreys would prohibit successful copulation (Hardisty, 1963). Lauterborn (1926) described communal spawning of *L. planeri* and *L. fluviatilis*, and speculated on the possibility of natural hybridization. Recent field observations on spawning lampreys are relevant to this question.

During March 1969, a single male brook lamprey was observed in the River Teme, in a redd occupied by three sexually mature river lampreys, although spawning activity was not taking place. In April 1969, in a tributary of the River Tywi, the two forms were associated during spawning. On a stream bed of pebbles, gravel and coarse sand, in water 30 to 60 cm deep at 11° C, a male and a female river lamprey, and one female and two male brook lampreys were nest building in the same redd. After an interval of 20 to 25 minutes, the redd was a saucer-shaped depression of about 23 cm diameter and 5 to 8 cm in depth. The male and female river lampreys then copulated*, resulting in the release of about 100 eggs. Immediately after copulating the lampreys recommenced redd building, which caused most of the eggs to be dislodged and to be swept downstream. Within a few seconds of the copulation of the river lampreys, a pair of brook lampreys copulated.

During a further period of about 25 minutes, the river lampreys copulated about a dozen times with the release of about 100 eggs each time. After each copulation the animals commenced redd building, and as a result of this continuous activity, few eggs remained in the nest. On several occasions two male river lampreys copulated simultaneously with the female river lamprey. At other times, pairs of both brook

* The word copulation is used in this context as a descriptive term for the intertwining of lampreys at the time of release of eggs and milt. It is not used to imply any intromission or internal fertilization.

lampreys and river lampreys copulated within seconds of each other, and frequently male brook lampreys attempted unsuccessfully to copulate with the female river lamprey.

Of the animals under observation in this area, 10 were captured and their lengths measured. The five specimens of *L. fluviatilis* varied from 19·2 to 21·8 cm and the five *L. planeri* from 10·3 to 12·3 cm. Informed local sources suggest that the river lampreys of the Tywi and its tributaries are much smaller than those of the Severn basin and both *L. fluviatilis* and *L. planeri* are referred to locally as 'brook lampreys'. For comparison, Plate I shows a male and a female river lamprey from the Tywi together with a representative pair from the Severn. It remains to be ascertained whether members of two species may occur in the same river at similar lengths, and may thus interbreed. The observations reported here suggest that even with reduced size differences between the species, interspecific copulation was not successful. However, it is possible that interspecific fertilization may occur under circumstances such as those described, where eggs and milt of both species were shed almost simultaneously. Although lamprey spermatozoa apparently remain active in freshwater for only about 50 seconds (Kille, 1959), the possibility of accidental hybridization must be considered.

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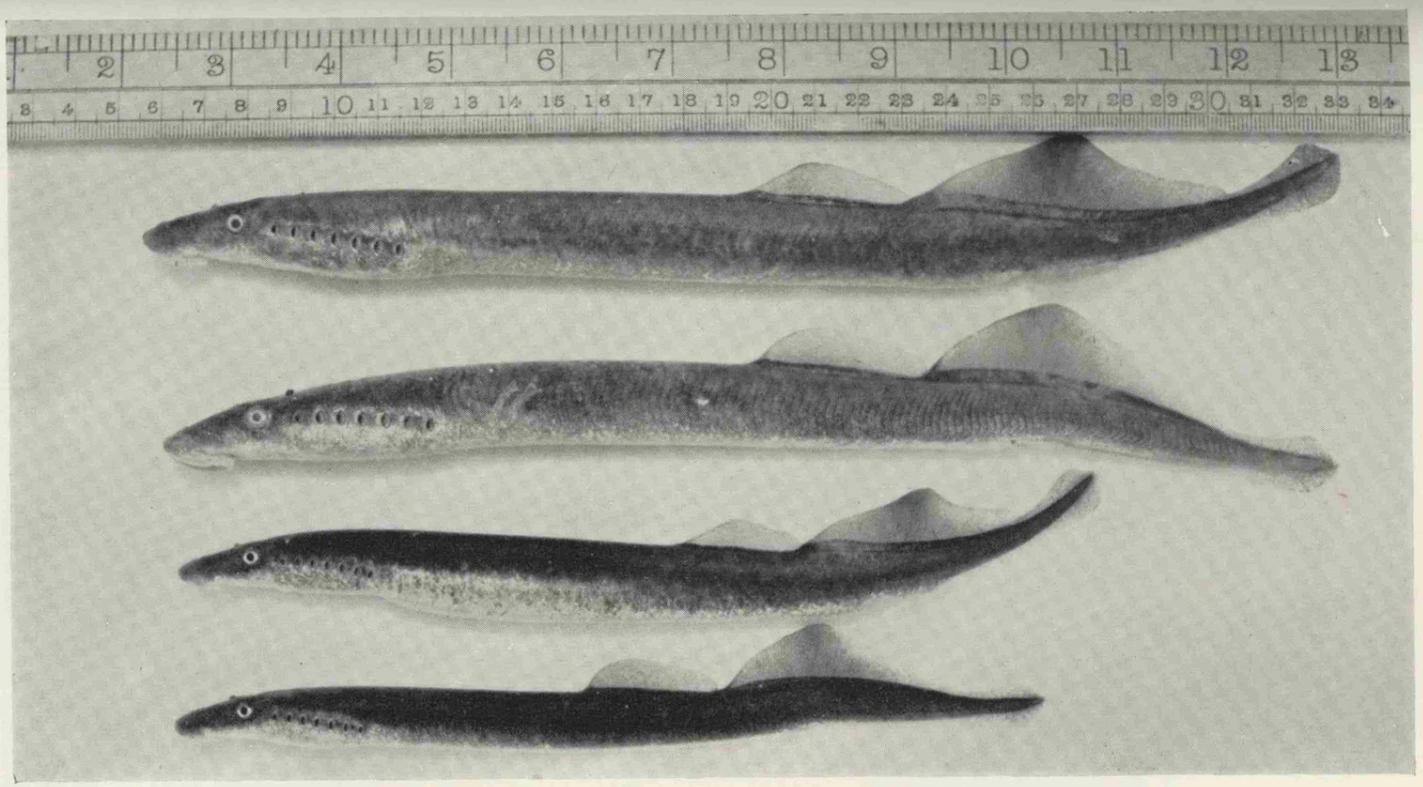

PLATE I

Comparison of representative specimens of *L. fluviatilis* from the Rivers Severn and Tywi.

From top to bottom:

Female—R. Severn
Male —R. Severn
Female—R. Twyi
Male —R. Twyi

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Larval growth in the river lamprey, *Lampetra fluviatilis*

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(With 1 figure in the text)

A series of 987 ammocoetes from the rivers Towy, Teme, and Taw have been identified as mainly *L. fluviatilis* (L.) on the basis of oocyte counts on female ammocoetes. The length-frequency distributions for this material differs from either *L. planeri* or *P. marinus* in showing only three modes in addition to the young of the year and the length distribution of the final mode coincides with the length range for 119 metamorphosing and macrophthalmia stages of *L. fluviatilis* that have been found at the same sites. These animals measured from 80–117 mm in length and weights varied from 0·76–2·28 g. Metamorphosis is believed to take place in late summer and early autumn when in the majority of cases, the ammocoetes are four and a half years old. The evidence that the non-parasitic *L. planeri* has a longer larval life than the closely related parasitic *L. fluviatilis* is thought to have some significance in relation to the evolution of the brook lamprey species.

Contents

	Page
Introduction	549
Methods	550
Identification of ammocoetes of *L. fluviatilis*	550
Length-frequency distributions	551
The age of the ammocoetes at metamorphosis	554
Discussion	555
Summary	557
References	557

Introduction

Although there have been many attempts to estimate the rate of growth and the duration of the larval period in the non-parasitic brook lamprey, *Lampetra planeri* Bloch (Hardisty, 1944; 1951; 1961*a*; Ivanova-Berg, 1931; Knowles, 1941; Lohnisky, 1965; Zanandrea, 1951; 1953) very few observations have been reported on the closely related parasitic species *L. fluviatilis* (L.). This is undoubtedly due to the difficulties in distinguishing ammocoetes of these two species. While it is true that especially in smaller streams, the ammocoete populations may consist exclusively of *L. planeri*, in most of the river systems that we have studied, this species is associated with *L. fluviatilis* and often also with ammocoetes of the sea lamprey, *Petromyzon marinus* L. (Hardisty, 1961*b*, 1969*b*).

After a detailed study of larval populations from a number of different streams it was considered that for ammocoetes of *L. planeri*, the duration of the larval period is five and a half years with metamorphosis in the late summer and autumn (Hardisty, 1961*a*), although

it was suggested that some larvae might transform either a year earlier or later. A similar range of variation in regard to age at metamorphosis has been observed in an experimental population of sea lamprey ammocoetes introduced into the Big Garlic River (Smith, 1966; P. J. Manion, pers. comm.). Some early observations made by Meek (1917) on ammocoetes from the river Tyne, believed to represent *L. fluviatilis* and later analysed by Hubbs (1925) suggest the presence of only three year classes. On the other hand, MacDonald (1959) working with rather small samples, estimated the larval period for this species as about five and a half years.

Methods

Ammocoetes and metamorphosed animals were collected by electric fishing in the rivers Teme Taw and Towy at various times throughout the year. The lengths of the animals were recorded after anaesthetization with MS 222 and length-frequency diagrams produced, using a sliding average of 7 mm. To assist in identification, a certain number of ammocoetes have been examined for oocyte numbers, by fixing and subsequently sectioning that part of the ovary lying at the anterior end of the mesonephric region. Counts were then made of the numbers of oocytes in transverse sections.

Identification of ammocoetes of *L. fluviatilis*

In his key for the identification of ammocoetes of British lampreys, MacDonald (1958) used as taxonomic characters, the distribution of pigment, the shape of the caudal fin and myotome numbers. Contrary to the findings of other authors who have failed to find significant differences in myotome numbers between *L. planeri* and *L. fluviatilis* (Hardisty, 1961*b*; Vlaydkov, 1955; Vladykov & Follett, 1958; Zanandrea, 1957, 1959) MacDonald (1958) gives figures for the latter species in the range 51 to 58 and in the former 61 to 69. In our own material we have not been able to confirm differences in the shape of the fins as described by MacDonald (1958).

In the sites on the river Teme where ammocoetes have been collected for the present study, larval sea lampreys (*P. marinus*) also occur and these have formed the basis of a separate publication (Hardisty, 1969*b*). Ammocoetes of *P. marinus* are readily distinguished from these of either *L. fluviatilis* or *L. planeri* by the characteristic shape of the caudal fin and by the presence of pigment extending from the tip of the body axis towards the fin margin (Hardisty, 1969*b*; Macdonald, 1958; Vladykov, 1950; 1960).

In the present work, identification of ammocoetes of *fluviatilis* has rested on three kinds of evidence:

(1) The presence in length-frequency distributions of a smaller number of age classes than are normally found in collections of *planeri* or *marinus* ammocoetes in the British Isles.

(2) The presence at the same sites of metamorphosing or macrophthalmia stages of *fluviatilis*, whose lengths are considerably below those normally found in similar stages of *planeri* and which correspond with the lengths of the oldest age groups in the ammocoete length-frequency distributions.

(3) Evidence from oocyte counts which gave values far beyond the range normally encountered in ammocoetes of *planeri* (Hardisty, 1961*b*; 1963; 1964).

Length-frequency distributions

The first collection of ammocoetes was made in the middle reaches of the river Teme in late July 1968. The length-frequency distribution for this material, consisting of 314 animals gave an exceptionally sharp definition of three modes at 43, 72 and 97 mm (Fig. 1). The young of the year may be represented by a very few animals with lengths between 18–26 mm. Both the modal lengths and the intervals between the frequency peaks agree very closely with previous observations on ammocoete growth rates, which have usually indicated seasonal length increments of 20–30 mm (Hardisty, 1961*a*; 1969*b*) and there seemed little doubt that the three major modes represent ammocoetes one and a half, two and a half and three and a half years old. The presence of only three modes in July and the fact that no ammocoetes larger than 124 mm were found suggested that this material consisted predominantly of *fluviatilis* ammocoetes.

TABLE I

Oocyte counts of ammocoetes from various sampling sites

Source of ammocoetes	Number of animals	Length range (mm)	Mean oocyte count ± standard error
R. Teme (Bransford Bridge)	11	70–80	72·8±3·5 (48–85)
R. Teme (Bransford Bridge)	23	95–120	71·7±4·5 (34–98)
R. Teme (Knightsford Bridge)	12	55–78	73·6±5·0 (49–100)
R. Honddu	50	60–100	25·6±1·4 (13–51)
R. Yeo	108	50–150	26·3±0·89 (7–53)

Confirmation was subsequently obtained by oocyte counts made on a total of 34 female ammocoetes selected from the second and third of the major frequency modes. Animals from the first mode could not be used since at this age the differentiation of the oocytes had not taken place. Sample oocyte counts were later made on a further 12 ammocoetes from another site on the Teme, where a collection was made in late May 1969 (Table I). In all three samples the mean values were similar (71·7–73·6) and the range of counts while varying widely from 34–100, exceeded 50 oocytes per section in no fewer than 41 out of the total of 46 animals. These figures may be compared with counts made on 160 ammocoetes of *planeri* from the rivers Honddu and Yeo, in which the range of counts was 7 to 53 with means of 25·6–26·3. In this species counts in excess of 50 were recorded in only four cases and none exceeded 53.

Additional confirmation comes from a consideration of the lengths of metamorphosing and macrophthalmia stages of *fluviatilis* that have been found in the course of the present work. Because of difficulties in distinguishing these stages in the life cycle of *planeri* and *fluviatilis*, published information on this subject is fragmentary and inconclusive. Zanandrea (1957, 1959) on the basis of only seven animals concluded that in Italy, metamorphosed and macrophthalmia of *L. fluviatilis* occur at lengths below the maxima attained

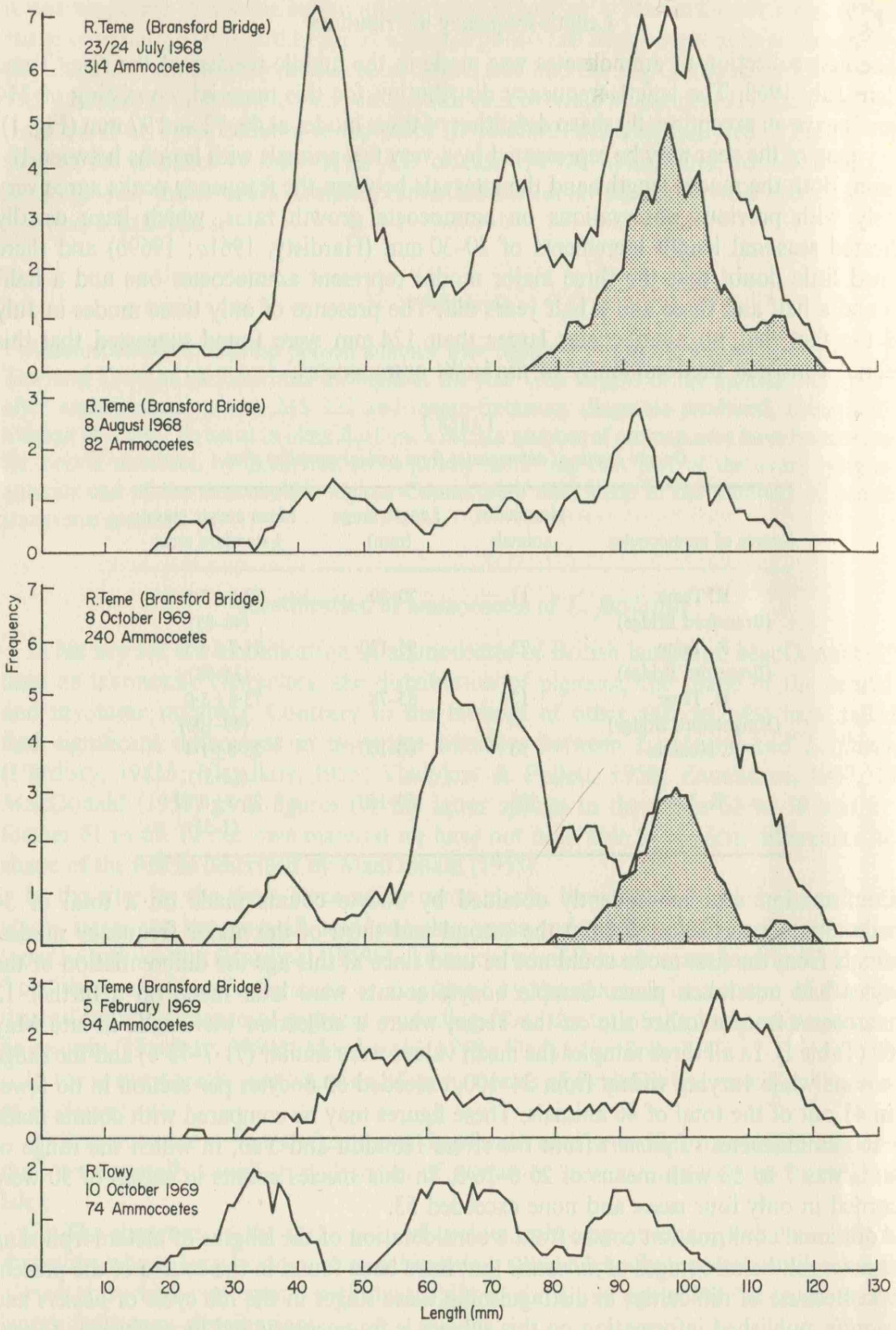

FIG. 1. Length-frequency distribution for ammocoetes and transformed specimens of *L. fluviatalis* from the rivers Teme and Towy at various periods throughout the year.
Stippled areas represent metamorphosed animals.

by *planeri*. Among the few specimens of these stages available to Weissenberg (1927) in his classical studies on the biology of the two *Lampetra* species, was one taken from the sea with a length of only 112 mm and Vladykov & Follett (1958) cited a specimen from England with a length of 108 mm. At the mouth of the Neva, Berg (1948) gives the mean length of newly metamorphosed *fluviatilis* as 120 mm with a range from 86–150 mm, although it is possible that some of the larger specimens might have been animals that had already begun to feed. Our own material consisting of 119 specimens taken from the rivers Teme, Taw and Towy during the period from late July 1968 to March 1969, and identified as *fluviatilis* by methods to be described in a future publication, shows a range of length from 80–117 mm with a mean of 96 mm. The weights of 43 of these animals varied from only 0·76–2·28 g with a mean of 1·45 g. Although populations of *planeri* adults are characterized by great local variability in length and weight, in the great majority of streams that we have studied the mean length has varied between 110 and 150 mm. In itself, the smaller size of the metamorphosed *fluviatilis* suggests a shorter larval period that that of *planeri* and evidence from the length-frequency distributions confirms that this reduced size at metamorphosis cannot be accounted for by a lower larval growth rate. Furthermore, it is significant that the distribution of lengths for the metamorphosed animals corresponds very closely with the third mode in the July material (Fig. 1).

A further collection of 82 ammocoetes was made at the same site on the Teme in August 1968, but during the last week in July there had been exceptionally severe flooding which seriously disturbed the conformation of the ammocoete beds. The length-frequency distribution for this material (Fig. 1) showed frequency maxima at 45 mm and 93 mm which are in good agreement with the first and third year class in the July material. There is also a suggestion of a group between 62–78 mm corresponding to the second year class in July. In both the July and August 1968 frequency curves these second year ammocoetes are poorly represented, perhaps reflecting either a small spawning population in 1966, or adverse conditions during the spawning season of that year. However, it should be noted that collections of ammocoetes made by the electrical method do not give a true representation of the numerical proportions of particular year classes, since there is always a bias toward the larger ammocoetes. Nevertheless, the small size of the 1966 class is borne out by further collections of 94 animals in the same area in February 1969 (Fig. 1) and for this material the frequency curve no longer shows a distinct mode for this group. As would be expected, the first year class (now nearly two years old) forms a group between 44–63 mm and judging from the median values, this class has progressed from about 43 mm in July 1968, to 54 mm in the following February. The presence in February of a group between 98–124 mm presumably now nearly four years old, raises the question of the timing of metamorphosis. The range of lengths of metamorphosed animals corresponded so closely with the third mode in July or August that it was reasonable to assume that this class would transform during the autumn of 1968. The length frequency distribution for February 1969, seems to imply that at least a proportion of this class did not in fact, metamorphose at that time, since the mode with a peak at 105 mm presumably represents ammocoetes which are now approaching the end of the fourth year of larval life.

Two further collections of ammocoetes were made at different sites in the Teme towards the end of May. At one of these sites (Knightsford Bridge) a sample of 12 female ammocoetes from the length group 55–78 mm gave oocyte counts of 49–100 (mean 73·6). In this material, consisting of 79 animals, the largest, presumably three years old,

form a group between 85–108 mm and a similar group is present in a collection of 78 animals from a second site with a peak at 91–95 mm. At lower lengths both curves are difficult to interpret, but it is possible that a group between 50–85 mm represented two year old ammocoetes. Very small numbers were present between 28–45 mm which could be assigned to the one year old class.

A further large collection of ammocoetes was made in the Teme in October 1969, together with transformed specimens (Fig. 1). In general, the length-frequency distribution shows good agreement with previous collections from this river, although the first and second modes are displaced slightly to the left, indicating a rather reduced growth in the one and a half and two and a half year class. This kind of variability in the growth of different year classes is by no means an unusual feature of ammocoete populations (Purvis, in press; Potter, 1970) and may be attributed to annual variations in temperature or rainfall, or to the movement into the area of animals from nearby sites, where nutritional conditions may be more or less favourable. These factors should also be borne in mind when considering the relation between the lengths of the metamorphosed animals in Fig. 1 and the range of length of the third frequency mode. Like the corresponding material collected during the previous year, the length-frequency curve for the transformed animals shows an approximately normal distribution, although in both instances it may be significant that there is a small but distinct shoulder at the upper end, which may represent a further age class. In the October 1969 material, the third ammocoete frequency peak is displaced to the right of the corresponding peak for the transformed animals and the average length of these ammocoetes is therefore greater than that of the macrophthalmia.

In the river Towy, at the majority of sites there has been evidence that both *planeri* and *fluviatilis* are present, and the frequency curves not only extend well beyond the normal upper range of length for *fluviatilis* ammocoetes, but show an increased number of modes. At one site, however, where a collection was made in August 1969 and where metamorphosed *fluviatilis* were found, the frequency distribution shows the characteristic pattern for this species, with three major groups present at ranges of approximately 18–49, 51–80 and 80–114 mm, which may be interpreted as age classes I–III (Fig. 1).

The age of the ammocoetes at metamorphosis

While the ammocoete length-frequency distributions leave little doubt that the majority, if not all the animals in the third mode, reach lengths similar to those of the transformed specimens at just over three years of larval life, there must still be an element of doubt as to what proportion of the ammocoete population actually transforms at this age. In this connection two possible alternatives might be considered:

(1) The persistence beyond the normal time of metamorphosis in late summer and early autumn of ammocoetes of metamorphosing length and the presence in the February material from the Teme of animals which appear to correspond in their length range to the three year old class of the preceding summer, might be taken to imply the possibility of metamorphosis continuing through the winter or in the following spring. Since this would be quite contrary to the evidence from all other species in the Northern Hemisphere, which invariably undergo metamorphosis in late summer and autumn, this possibility can probably be safely excluded. It should also be noted that specimens of *fluviatilis* in the earliest stages of transformation have so far only been recovered within the restricted

period from the end of July to the end of August, although completely transformed animals have been found up to the end of October and in February and March. During the intervening winter months the condition of the rivers has made electrical fishing impracticable.

(2) A more probable explanation and one which is consistent with the data, would be that a large proportion and perhaps the majority of ammocoetes, fail to metamorphose when they are three years old and that their transformation is delayed until the following summer. The existence of mixed age groups in transformed populations of non-parasitic species has already been suggested for *L. planeri* (Hardisty, 1961*c*) and for *Ichthyomyzon fossor* (Purvis, in press) and the same situation has also been demonstrated in the landlocked sea lamprey, *P. marinus* of the Great Lakes Basin (Smith, 1966; Stauffer, 1956; 1960; 1962; Thomas, 1962). Two consequences must follow from this interpretation. In the first place, the third frequency mode in material collected before the general onset of metamorphosis must in fact represent two age classes of three and four year old animals. Secondly, it must follow that there is little if any further growth in length during the fourth and final year of ammocoete life, since they have already reached lengths comparable to those of the metamorphosed animals by the autumn of the previous year.

Belief in a final "rest year" before metamorphosis has been widely canvassed by North American authors (Gage, 1928; Leach, 1940; Churchill, 1945; Horn & Bailey, 1952) and has been based on the presence in ammocoete populations of animals larger than those actually undergoing transformation. In this connection, it is interesting to find that a rather similar situation has recently been described in the Australian genus *Mordacia* (Potter, 1970) where there is evidence that in certain areas very little further increase in length occurs during the final year of larval life and that as a result the modes representing the final and penultimate year classes often tend to fuse to such an extent that they give the appearance of a single mode.

Discussion

Even on the assumption that in *fluviatilis* a majority of the larval population transforms at a maximum age of four and a half years, this implies a considerably shorter larval period than in the closely related, non parasitic *L. planeri*. In the latter species, investigation of length-frequency distributions for the ammocoete populations of a number of British streams has generally revealed the presence of up to five frequency modes in addition to the young of the year (Hardisty, 1951; 1961*a*). This is in contrast to the *fluviatilis* populations, where only three such modes have been observed. On this basis, therefore, the duration of the larval period in the two species might be expected to differ by two years. From a consideration of length-frequency distributions in relation to the lengths of adult spawning populations, it was believed that for the majority of *planeri* ammocoetes, the duration of the larval period was five and a half years, although a smaller proportion might metamorphose at four and a half years, or six and a half years, the numbers involved probably varying in the two sexes and from one season to another (Hardisty, 1961*a*; 1961*c*). However, the investigations on *fluviatilis* which have been reported here, show that the larval period may easily be underestimated, if too much reliance is placed on an apparent correspondence between the length distribution of metamorphosed animals and the final mode in the larval length-frequency curves. If indeed, a "rest year" of retarded growth is a normal feature of ammocoete populations, this would not be apparent

from the length-frequency distributions, unless representative samples of both ammocoetes and metamorphosing animals were available immediately before and after the time of transformation. For these reasons therefore, it may well be the case that previous estimates of larval life in *planeri* should be revised by the addition of a further year, making the average duration of the ammocoete period six and a half rather than five and a half years.

This interpretation is also supported by a consideration of the rates of larval growth in the two species. In *fluviatilis*, the position of the modes in length-frequency curves and their separation, indicate average annual growth increments of about 20–25 mm, which are quite similar to those previously observed in *planeri* populations from the River Yeo (Hardisty, 1961*a*) where the average length at metamorphosis was estimated as about 147 mm. Comparing the latter with the average length of metamorphosed *fluviatilis* (96 mm), it seems clear that on the assumption of a similar linear rate of growth, the *planeri* ammocoete could hardly reach transforming lengths with less than two additional seasons of growth.

These observations on the larval cycle of *L. fluviatilis* have some relevance to the much debated problems of the origins of the non-parasitic lampreys and the relationship between paired species such as *L. planeri* and *L. fluviatilis*. The view has been widely held that the non-parasitic species have evolved from the parasitic forms by a kind of paedomorphosis (Hardisty, 1960; 1965*a*,*b*; 1969*a*; Leach, 1951; Young, 1962; Zanandrea, 1956) in which sexual maturity has been accelerated to such an extent that it is virtually superimposed on metamorphosis and that, as a result, adult life has been reduced to a short period of six to eight months. The evidence presented here for earlier metamorphosis in *L. fluviatilis* compared with *L. planeri* makes possible a rather different interpretation; that the latter is a form in which metamorphosis has been delayed relative to the position in its parasitic ancestor. Both the curtailment of adult life and the apparently earlier sexual maturity would, from this viewpoint, be seen as a consequence of the postponement of metamorphosis by at least one, and more probably, two years. In view of these findings, it would be very interesting to have similar information on other paired species, although the close similarities in their ammocoete forms poses formidable taxonomic problems. However, it is significant that recent accounts of the biology of the Southern Hemisphere genus *Mordacia* have shown that in this case also, the non-parasitic species *M. praecox* at the time of metamorphosis, is larger than the corresponding stage in the parasitic form *M. mordax*, and consequently may be supposed to have a longer larval life (Potter, 1970).

Although we have no precise information on the duration of the adult phase in the life cycle of *L. fluviatilis*, indirect evidence suggests that, for the majority of the population, this is probably about two and a half years from the time of metamorphosis. Since the downstream migration of the macrophthalmia probably occurs about six months after the onset of transformation (Hardisty, Potter & Sturge, 1970) and the upstream migration, at a similar period before spawning, the feeding phase would be about 18 months, which is in broad agreement with the views of Berg (1948) and Zanandrea (1959). Assuming a maximum larval period of four and a half years, this would give for *fluviatilis*, a total life span of seven years, and if, as suggested earlier, the average larval period in *planeri* is six and a half years, the total life span in this species would be identical to that of the river lamprey. If these arguments are valid, the evolution of the non-parasitic species has not involved any change in the total time required to reach full sexual maturity and from this point of view it would be difficult to regard the brook lamprey species as a paedomorphic form.

Summary

An examination has been made of the length-frequency distributions of 987 ammocoetes of *L. fluviatilis* from the rivers Teme, Taw and Towy.

The identity of these ammocoetes has been established by oocyte counts on female ammocoetes. These gave mean values of 71–73 compared with values of 25–26 in ammocoetes of *L. planeri.*

In most of the length-frequency curves only three year classes are present, in addition to the young of the year. This is contrasted with the situation in *L. planeri* and *P. marinus* where at least five year classes are normally present.

Metamorphosing and macrophthalmia stages of *L. fluviatilis* have been found with lengths from 80–117 mm, which is far below the usual range of length of metamorphosing *L. planeri* in British streams. This length distribution corresponds closely with the final mode in late summer.

The persistence of ammocoetes with lengths similar to those of the transformed animals, in frequency curves for periods beyond the normal time of metamorphosis, must indicate that many if not all the ammocoete population do not transform until they are about four and a half years old. If this is the case the frequency curves show that little or no increase in length can occur during this final period.

This evidence that *L. fluviatilis* has a shorter larval period than the closely related *L. planeri* suggests that delayed metamorphosis, rather than precocious sexual maturity may be the significant feature in the evolution of the non-parasitic species.

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Observations on the morphology, behaviour and salinity tolerance of downstream migrating River lampreys (*Lampetra fluviatilis*)

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(With 1 plate and 4 figures in the text)

Downstream migrating *Lampetra fluviatilis* were caught at night in elver trawls from the lower reaches of the River Severn during the high spring tides of 1970, 1971 and 1972. The length and weight-frequency curves indicated that in 1970 and 1972, the populations consisted mainly of animals of single year class. On the basis of previous estimates of the duration of larval life, they were thus probably five years old, while a small group of larger animals may have been one year older. In 1971, there was a greater proportion of larger animals, several of which differed in their weight/length relationship from others in this sample and from those of 1971 and 1972, possibly reflecting differences in feeding conditions during larval life. Laboratory studies on the activity rhythms of downstream migrants showed that emergence from the substrate and swimming was primarily nocturnal, with an initial large peak in free-swimming activity at the onset of darkness and a smaller peak at the transition from the dark to the light phase. During the light period, these animals showed a significant preference for burrowing or lying in regions of gravel and pebbles. Downstream migrants examined in May/June 1972 were capable of being acclimated to full strength sea water (34–35‰) and a large proportion (80%) survived for three weeks or more after direct transfer. Parallels are drawn between the biology of this stage in the lamprey life cycle with that of similar stages in salmonid fishes.

Contents

	Page
Introduction	365
Materials and methods	366
Results	367
Field data	367
Lengths, weights and body proportions	367
Emergence behaviour, swimming activity and habitat selection	372
Salinity tolerance	373
Discussion	374
References	378

Introduction

Few studies have been reported on the downstream migrant phase of anadromous lampreys. This is due, in most species, to the shortage of information on the precise timing of the migration, the ecological and physiological factors involved in its initiation, and the habitats occupied during this stage of the life cycle. The few published observations on downstream migrating *Lampetra fluviatilis*, indicate that they enter the estuary during

the late winter and spring (Weissenberg, 1925, 1927; Bahr, 1952), some five to eight months after the initiation of metamorphosis (Hardisty, Potter & Sturge, 1970). On the basis of distribution records and salinity tolerance experiments, Bahr (1952) suggested that these juvenile adults are adapted to living in areas of reduced salinity, although both he and Morris (1972) were successful in acclimating some individuals to salt water concentrations approaching 35‰.

During the last three years, small, apparently fully metamorphosed, *L. fluviatilis* have been caught in elver trawls from the River Severn. These samples provide data on the timing of the downstream migration and also on the pattern of activity throughout the day. The lengths, weights and proportional body measurements of these animals are compared with those obtained from stages caught earlier in metamorphosis (Hardisty, Potter & Sturge, 1970) to give information on morphological changes during this process. In order to explore the daily pattern of activity and possible ecological requirements, their times of burrowing, emergence and swimming were recorded in aquaria containing different substrates. They were also subjected to various salinity regimes to ascertain their ability to tolerate these conditions.

Materials and methods

Downstream migrant *L. fluviatilis* were caught in the River Severn with a fine-mesh trawl during the spring of 1970, 1971 and 1972. The mouth of the trawl, 5 ft square, was towed vertically just below the surface approximately 40 ft behind a motor launch. The sampling procedure, which involved holding the boat against the reversed flow associated with the bore or tidal wave that passes up the Severn on the spring high tides (Bassingdale, 1943), was designed to catch elvers migrating upstream under these conditions. Trawling was restricted mainly to the stretch of river between Gloucester and Tewkesbury, although, in 1972, successful expeditions were also undertaken in a 5–6 mile reach below Gloucester in an estuarine region of fluctuating but low salinities. Trawling in the main sampling area generally lasted for the duration of flow reversal, a period of about 1 hr.

The animals were brought back to the laboratory where, after anaesthetization in MS 222 (Sandoz), their length, weight and body intervals were measured in the manner described by Potter, Lanzing & Strahan (1968). In 1972, animals were placed in 2 glass aquaria (48 × 15 × 15 in) containing equal areas of 4 different substrates. The diameter of most of the particles in the fine, medium and coarse sand areas were approximately 0·3–0·6 mm, 1–2 mm and 2–7 mm respectively, while the fourth substrate consisted of gravel (6–12 mm) and pebbles. The relative positions of the 4 substrates were arranged differently in the 2 aquaria, which contained 11 and 16 animals respectively. These experiments were carried out in a constant temperature room (8°–9°C), with a light/dark cycle paralleling field conditions. The lighting was provided by overhead fluorescent tubes, direct light being prevented from falling on the water surface by opaque aquarium covers. The average light intensity on the side wall of the aquaria was 1·1 ft candles. After they had been left undisturbed for at least 4 days, the numbers of animals that had emerged in each aquarium, and were either lying on the substrate or swimming, were counted at hourly intervals throughout three 26 h periods. At the end of each trial the location of the animal i.e. burrowed or unburrowed and in or on which substrate, was recorded during light conditions (at 11.00 hrs) by careful removal of each animal.

Preliminary salinity tolerance experiments on downstream migrants, involving both gradual acclimation and direct transfer to 100% sea-water (34–35‰) were carried out between March and May of 1970 and 1971, both on animals held in the laboratory from the commencement of metamorphosis and on others recently taken from the river. In May and early June 1972, more

detailed studies were performed under the laboratory regime described above for the behaviour experiments. The acclimation experiments of 1972 involved placing 15 animals for 4 days in 33‰ and then for a further 4 days in 67% before transfer to 100% sea-water (solutions monitored by a chloride meter). Fifteen animals were also used for the experiments in which lampreys were transferred directly from fresh-water to 100% sea-water. The behaviour of the animals was observed at frequent intervals and, if applicable, the time of death noted. The survival results are based on a 3 weeks duration in 100% sea-water with 10 animals being held in fresh-water as controls. In none of the salinity experiments in 1971 and 1972 were animals transferred to salt water until after they had been held in the laboratory for at least 3 weeks.

Results

Field data

Examination of elver trawl catches made on the day and night high spring tides of 5–9 April 1970, showed that the upstream elver migration occurred on all tides at this time but was more pronounced at night. The activity of *L. fluviatilis* in this part of the river appeared, however, to be nocturnal. Thus, during these five days, 37 small metamorphosed lampreys (Plate I) were caught, all of which were taken on tides occurring after sunset. The only nocturnal high tide on which no lampreys were caught was that of 7 April, even though the trawl yielded an average elver catch.

On three days (27, 28, 29 March) of the spring tides of 1971, a similar pattern of nocturnal activity was observed, with no animals appearing in trawls during the day. However, the number of animals caught in each nocturnal sample at this time was greater than that of any other single catch from the same region during either 1970 or 1972 with 20, 20 and 16 animals being obtained on the three successive nights. Again, in 1972, all 21 individuals captured from this area between 30 March and 15 April were taken at night, although, in a sample of 28 animals from two days trawling in the estuary, two animals were obtained during daylight. During 1972, a further three days' sampling in the lower reaches of the Severn on 12, 13 and 14 May produced five more individuals showing that the migration had not been fully completed at this time.

One interesting feature of the contents of the elver trawls was the capture of eight adult lampreys, significantly larger than downstream-migrant *L. fluviatilis* but considerably smaller than the vast majority of those adults caught further upstream near or on the spawning grounds which are considered to have embarked on their migration in the previous autumn (Plate I). The most noticeable feature of these *L. fluviatilis*, which ranged in length from 175 to 224 mm, is that in their silvery colouration and general appearance they resembled downstream migrants much more closely than the typical large upstream migrants. In comparison with this latter group, they also had relatively sharper teeth and a larger intestine. However, after being held in the laboratory for seven weeks, until early June, the teeth became blunter, the intestinal diameter was reduced and the gonads reached an advanced stage of maturity with the oocytes displaying a well developed granulosa layer and the testis lobules containing spermatids.

Lengths, weights and body proportions

The range in lengths and weights of downstream *L. fluviatilis* caught in the elver trawls during 1970, 1971 and 1972 were respectively; 88–125 mm, 0·82–2·39 g; 84–133 mm, 0·76–2·49 g, and 83–120 mm, 0·64–2·17 g.

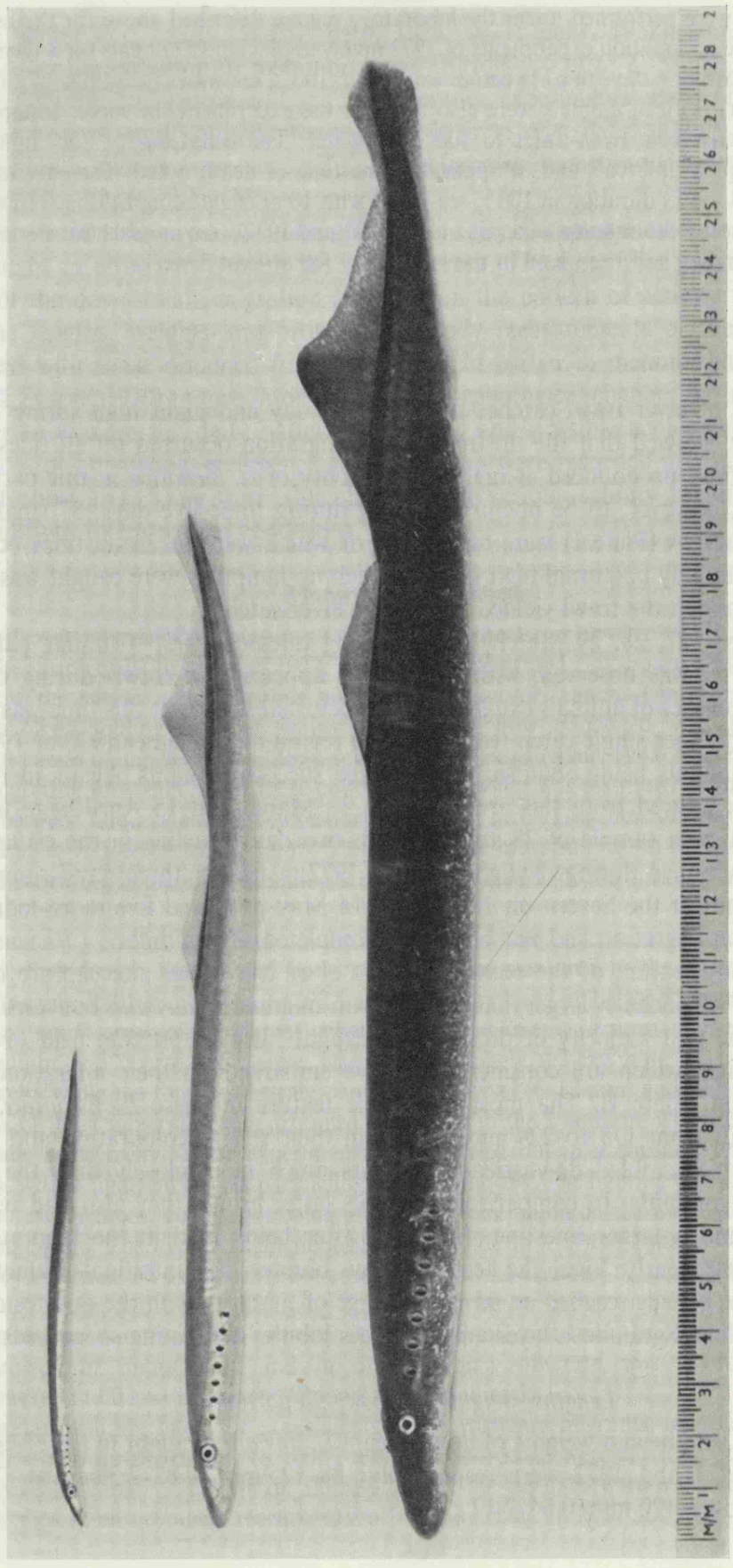

PLATE I. Postmetamorphic stages of *Lampetra fluviatilis* caught in the River Severn during April 1970. Top: downstream migrant; middle: spring upstream migrant; bottom: typical autumn upstream migrant. First two stages caught in elver trawls from the lower reaches of the Severn while the third was collected from a spawning area in the upper regions of one of its tributaries (R. Teme).

All three length-frequency curves comprise one large peak followed by one or more smaller peaks (Fig. 1), the mid-point of the "trough" between the two regions being sufficiently clear to permit estimates of this value in the three successive samples as 112, 106, and 108 mm. This close similarity is paralleled by the mode of the clearly defined initial peak which occur at 97·5, 96 and 93 mm respectively. The samples differ, however, in the proportion to the right of the mid-point of the "trough", with values of 12% in 1970, 49% in 1971, and 20% in 1972.

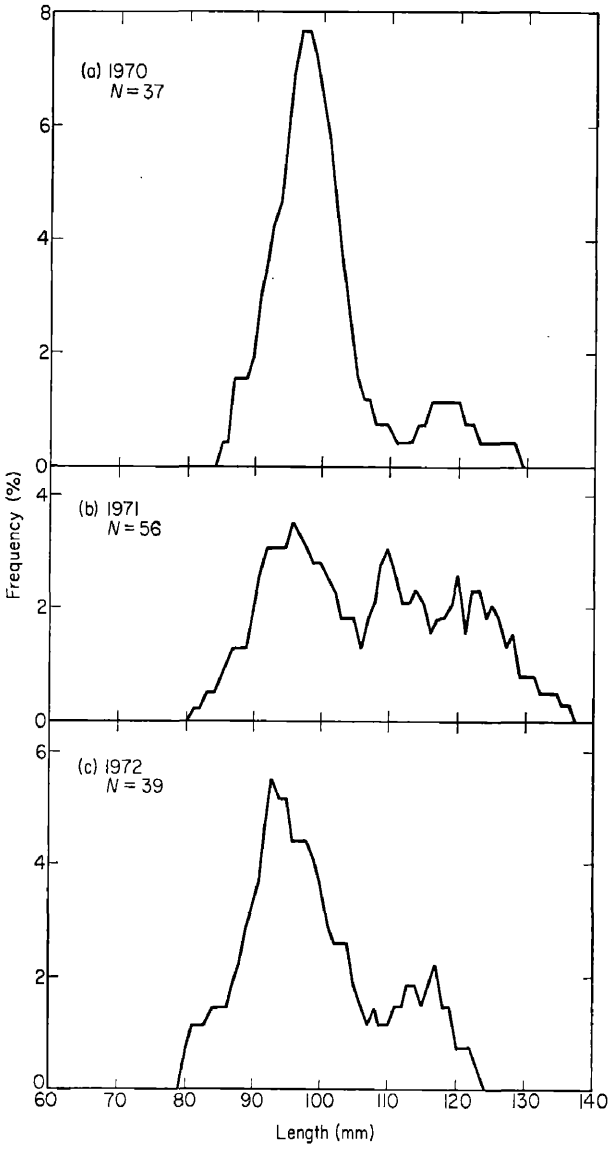

FIG. 1. Length-frequency curves for downstream-migrant *Lampetra fluviatilis* caught in elver trawls in the River Severn during (a) April 1970, (b) March 1971 and (c) March/April 1972. Data smoothed by moving averages of 7 mm. *N*, Number of animals.

As in the length-frequency data, the first mode in the weight-frequency curves is the most clearly defined and occurs at 1·00 g in each sample (Fig. 2). In 1970, the bimodal weight-frequency curve with a small second peak closely resembles that of the corresponding length-frequency curve (cf. Figs 1(a) and 2(a)), and a similar but slightly less

marked correspondence can be seen in the 1972 sample (cf. Figs 1(c) and 2(c)). However, in 1971, despite the presence in the weight-frequency curve of a well defined first peak, there is no conspicuous broad area comprising one or more smaller peaks as is the situation in the length-frequency curve (cf. Figs 1(b) and 2(b)).

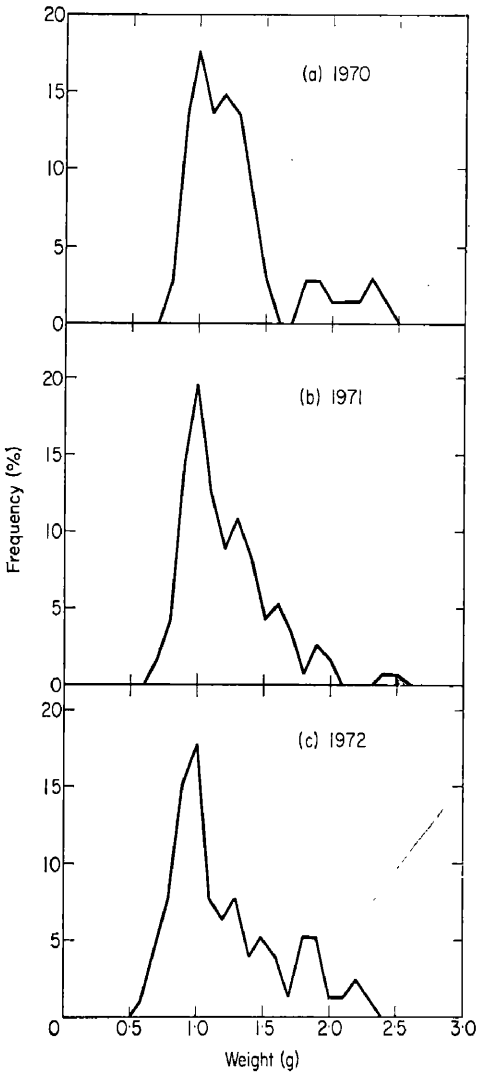

FIG. 2. Weight-frequency curves for the same downstream-migrant *Lampetra fluviatilis* as in Fig. 1. Data smoothed by moving averages of 0·2 g.

The regression coefficient and intercept for log weight on log length are similar for the downstream migrants caught in 1970 and 1972, each population comprising a relatively homogeneous group of animals (Fig. 3(a), (c); Table I). In the 1971 sample, however, the animals fall into two different groups with respect to the position of the log weight/log length regression line (Fig. 3(b); Table I), as would be anticipated from the weight and length-frequency data already discussed. After separating the 16 animals that are conspicuously different in 1971, the rest of the population can be seen to have a similar regression for log weight on log length as in the previous and following years (Table I).

Analysis of variance showed that there was no significant difference between the regression coefficients for weight on length of the 1970 and 1972 populations and the

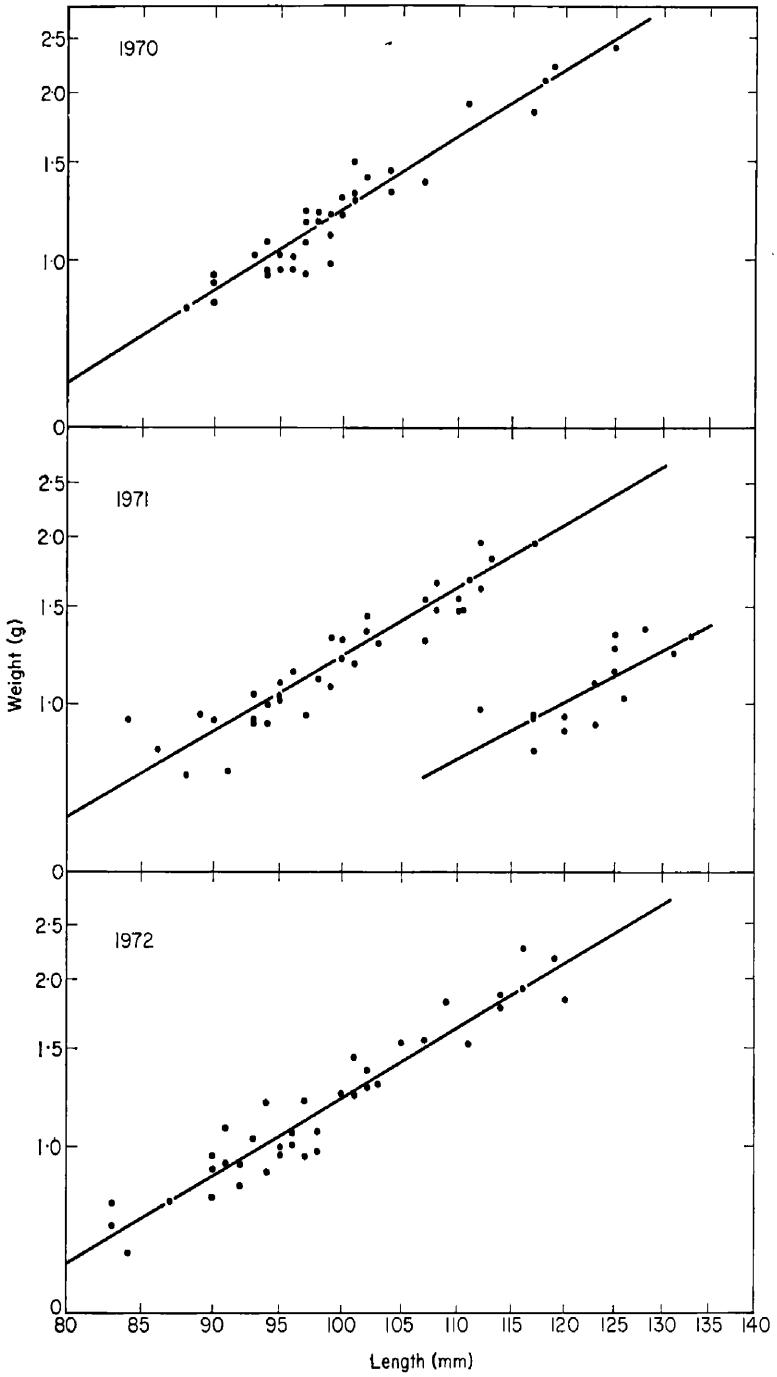

FIG. 3. Regression of log weight on log length (line obtained by method of least squares) for downstream-migrant *Lampetra fluviatilis* caught in 1970, 1971 and 1972.

main group in 1971, even at the 25 % level. After adjusting to produce parallel slopes the same situation was also found with respect to the intercepts. However, although the regression slopes for the main group in 1971 and that of the other 16 animals in this year also did not differ significantly, their adjusted intercepts were highly significantly different ($P<0{\cdot}001$).

The proportional body measurements of samples from 1970, 1971 and 1972 are seen in Table II, which also contains data for animals measured on 1 December 1969, i.e. at a time equivalent to several months earlier in metamorphosis.

TABLE I

Relationship between the log weight and log length of downstream-migrant ***Lampetra fluviatilis*** *caught in the River Severn during 1970, 1971 and 1972*

1970	$\log W = -12{\cdot}114 + 3{\cdot}174 \log L$
1971	I $\log W = -11{\cdot}225 + 2{\cdot}981 \log L$
	II $\log W = -10{\cdot}575 + 2{\cdot}692 \log L$
1972	$\log W = -11{\cdot}644 + 3{\cdot}072 \log L$

W, Weight (g); L, length (mm).
The equations I and II refer respectively to the upper and lower regression lines in Fig. 3(b).

TABLE II

Lengths, weights and body proportions (mean±1 standard error of the mean) for metamorphosing (from Hardisty, Potter & Sturge, 1970) and downstream-migrant stages of ***Lampetra fluviatilis***

Stage	Total length (*T.L.*)	$\frac{d}{T.L.}$	$\frac{d\text{–}o}{T.L.}$	$\frac{O}{T.L.}$	$\frac{B_1\text{–}B_7}{T.L.}$	$\frac{Lpd}{T.L.}$	$\frac{a\text{–}c}{T.L.}$	$\frac{D}{T.L.}$	Weight	Number of animals
Metamorphosing	100·3	4·9	7·7	2·7	9·8	22·7	29·6	5·9	—	45
1 Dec. 1969	±1·31	±0·11	±0·13	±0·14	±0·049	±0·18	±0·24	±0·059		
April 1970	100·11	5·13	7·89	2·84	9·05	22·40	29·50	5·45	1·257	37
	±1·38	±0·07	±0·07	±0·05	±0·06	±0·21	±0·27	±0·07	±0·062	
March 1971	106·79	4·79	7·55	2·70	9·22	22·29	29·92	5·35	1·217	56
	±1·73	±0·09	±0·07	±0·05	±0·07	±0·31	±0·18	±0·06	±0·045	
March/April 1972	99·97	5·09	7·92	2·79	9·29	21·9	29·19	5·58	1·216	39
	±1·77	±0·08	±0·09	±0·07	±0·08	±0·35	±0·20	±0·09	±0·072	

a–c, Tail length; B_1–B_7, length of branchial region; *d*, disk length; *d–o*, length of preorbital region; *D*, depth of body; *Lpd*, length of posterior dorsal fin; *O*, diameter of eye. Body proportions are expressed as percentage of the total length.

Emergence behaviour, swimming activity, and habitat selection

The hourly counts of the numbers of animals swimming or resting on the substrate clearly shows that activity is much greater in darkness (Fig. 4). The majority of animals burrowed during the light period, and of the few that remained above the substrate surface, only one was observed swimming after the lights had been on for three hours. In this circadian rhythm of emergence, some animals came out of the substrate before the lights went off, but this did not result in any marked swimming activity. When the lights went out more animals started to emerge with the result that at 22.00 hours 44% were above the substrate, a value five to six times greater than the percentage that were not burrowed at 11.00 hours. Swimming activity was greatest at 22.00 hours, after which it steadily declined until just before the lights came on when there was a small transient increase in the number of animals swimming. The pattern was similar in all three observed periods in the two aquaria but activity was slightly greater in the 16 animals caught in the estuary than in that of 11 individuals obtained from the river above Gloucester. Although a circadian rhythm of swimming activity was maintained for a considerable length of time

in the laboratory, the number of animals that burrowed in the light declined after the first few weeks.

An analysis of the locations of the lampreys in the aquaria showed that there was a marked preference for the pebble area (Table III) in to which the animals often burrowed in groups. The heads of the lampreys were occasionally seen projecting above the substrate surface, a position which could also occasionally be induced by turning the aerator off for a period. The unburrowed animals also preferred this substrate and could generally be observed lying between the pebbles. In full-strength sea water, the burrowing behaviour was far less marked and was not reacquired by spawning-run animals.

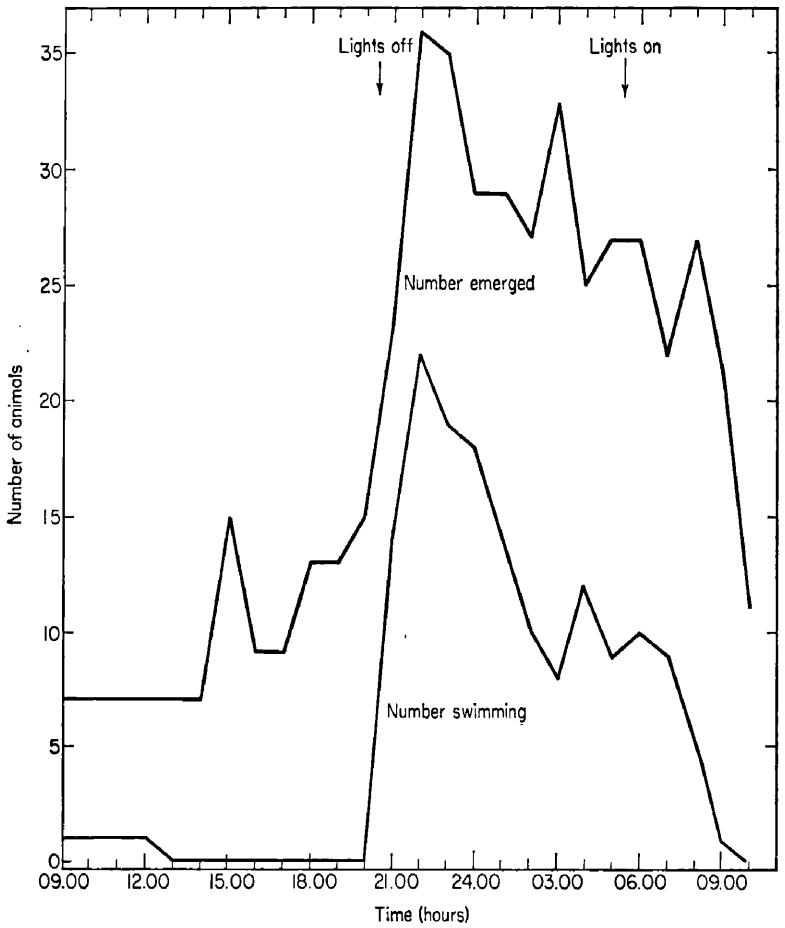

FIG. 4. The pattern of emergence and swimming activity of downstream-migrant *Lampetra fluviatilis* in aquaria exposed to a light/dark cycle. The graph shows the pooled hourly recordings on 27 animals taken during three separate 26 hour periods.

Salinity tolerance

Preliminary experiments in 1971 on the salinity tolerance of downstream-migrant lampreys, showed that by March, they had apparently developed the ability to osmoregulate in sea-water. Thus, in a sample of 18 freshly caught lampreys, acclimated to 100% via approximately 33 and 67% strength sea-water, 17 were still living at the end of three weeks. In the case of the direct transfer of 12 animals to full-strength sea water, nine were surviving at the end of three weeks, an appreciably higher percentage than that found in similar experiments (approximately 50%) performed on animals held in the

TABLE III

*Distribution of downstream-migrant **Lampetra fluviatilis** in aquaria containing four different substrates*

	Partially or fully burrowed				Unburrowed			
Substrate	Gravel and pebbles	Coarse sand	Medium sand	Fine sand	Gravel and pebbles	Coarse sand	Medium sand	Fine sand
Number of animals	52	1	4	6	7	1	0	0

The results were obtained by recording during the light period (at 11.00 hours) the location of the 27 animals after the completion of each of the three replicate experiments employed for the results shown in Fig. 4.

laboratory for seven to nine months from soon after the commencement of metamorphosis.

In the closely monitored experiments of May/early June 1972, carried out on samples recently caught in the field, all the 15 individuals acclimated for four days to 33 and 66 % sea-water, survived for at least three weeks in full-strength sea-water. After direct transfer to full strength conditions, 12 of the 15 animals were surviving at the end of three weeks, the three deaths all occurring within the first 72 hours of the experiment.

In the direct transfer to 100 % sea-water, of 25 animals held in the laboratory from soon after the commencement of metamorphosis until May/early June 1972, a higher proportion (70 %), compared with the preliminary experiments of 1971, were still alive at the end of three weeks. This was probably due to the reduction of light stress by the provision of cover in the holding tanks during the autumn and winter.

None of the control animals died during the experimental period and most of the lampreys in 100 % sea-water survived for at least a further month. Any subsequent deaths are attributable to an inability to maintain this migrant stage successfully in small aquaria over a long period.

A characteristic feature of the first 48 hours of the direct transfer experiments, was the development, in several animals, of a ventral bulge, most marked in a position corresponding to the first third of the intestine. The ventral protrusion was greatest in the three animals that died, whereas it gradually decreased in the other individuals and had disappeared by the end of the first week.

Eight animals returned to fresh-water after a period of four weeks in full strength sea-water were still surviving three weeks later.

Discussion

Since the trawl was held against the direction of the bore so as to catch elvers migrating upstream, it could have been collecting lampreys swept up from the estuary. However, it seems likely that the effect of the tidal wave is probably to circulate the animals in the water after they have emerged from the substrate at night. Because no recently metamorphosed lampreys were caught in the river during the day and only two were taken by daylight from the estuary, the bore alone apparently causes insufficient disturbance to stimulate emergence.

Due to the restriction of elver trawling to the period between late March and the middle of May, it is impossible from these data alone to ascertain precisely the overall timing of the downstream migration. Yet, the capture in the Severn of numbers of apparently fully metamorphosed *L. fluviatilis* in trawls from the lower reaches of the river and the upper part of the estuary during the above period in three different years, showed that a marked migration occurs at this time, thus paralleling the situation in other parts of Europe (Weissenberg, 1925, 1927; Bahr, 1952). That peak migration occurs in the Severn during the spring, and that a massive movement is unlikely to take place much earlier, is supported by other evidence. Thus, numbers of *L. fluviatilis* have been taken in March by electric fish shocker from sites on the River Teme, a tributary of the Severn, but have never been found in the same areas after mid-April. Furthermore, the opening of the fore-gut, so essential for the swallowing process involved in sea-water osmoregulation may not, at least in some individuals, have been completed by February (Hardisty *et al.*, 1970) or even, exceptionally, May (Weissenberg, 1927). Finally, the change from larval to adult haemoglobins, which may be essential for coping with the activity involved in migration and a predatory habit, is still in a transitional phase in early January (Waterfield, pers. comm.).

The time of capture of downstream migrant *L. fluviatilis* in this study parallels that of the major movement in some landlocked populations of *P. marinus*, the only other holarctic lamprey for which there is detailed field information. Thus, Applegate (1950) obtained large numbers of downstream migrants in the Ocqueoc River during March and April, a movement apparently correlated with flood conditions resulting from the spring break-up. Water flow has also been shown to exert a marked influence on the timing of the migration in the Southern Hemisphere species, *Mordacia mordax* (Potter, 1970). Although spring floods and increasing water temperatures may also be major factors involved in the initiation of the seawards movement of *L. fluviatilis*, the analogy with landlocked *P. marinus* should not be pressed too far. In addition to the very marked spring migration observed by Applegate (1950), other populations of this landlocked lamprey migrate mainly in the autumn (Wigley, 1959; Manion & Stauffer, 1970; Beamish & Potter, 1972). This situation is, however, unlikely to be paralleled by *L. fluviatilis* since *P. marinus* commences transformation in July rather than August (Wigley, 1959; Manion & Stauffer, 1970; Hardisty *et al.*, 1970) and completes its metamorphosis more rapidly (Beamish & Potter, 1972).

The mainly nocturnal downstream movement observed in migrating *L. fluviatilis* has also been reported by Gritsenko (1968) for comparable stages of *Lampetra japonica*. The bimodal activity rhythm (Fig. 4) parallels that observed in upstream migrants, both in their pattern of movement during the night (Wikgren, 1953; Skidmore, 1959) and in their heart and respiratory rates (Claridge, Potter & Hughes, in prep.). That lampreys at other stages of their life cycle are also predominantly nocturnal is further borne out by the field studies on ammocoete movement by Gritsenko (1968) and Long (1968). The pattern of activity rhythms also resemble those recorded in mice (Shillito, 1966), which suggests a similar physiological basis in both lampreys and higher vertebrates. Since work on birds and mammals has implicated the pineal in locomotory rhythms, and shown that there is a circadian rhythm of 5-Hydroxytryptamine production (Quay, 1964; Gaston & Menaker, 1968), the well developed pineal of lampreys (Eddy, 1972) may likewise be involved in circadian activity rhythms.

The marked preference for the gravel and pebble substrate is difficult to interpret in

terms of the environment in the lower reaches of the river where the substrate consists mainly of "mud" or "sand". The preference for a substrate of large particle size may be related to the change to tidal gill ventilation during metamorphosis, although they are still able to burrow in substrates of smaller particle size. This view is supported by the observations of Pickering (pers. comm.) who has found during April the greatest density of downstream migrants in the gravel and pebble areas of rivers in the Lake District.

A major factor in the timing of the downstream migration of anadromous lampreys, must be their ability to survive and osmoregulate in salt water. In this context, the experiments carried out in May/June 1972 on 15 downstream migrants recently collected from the field showed that they were most likely to survive in 100% sea-water if they were first acclimated through 33% and 67%. However, even under the extreme conditions of direct transfer to full-strength saline conditions, a large percentage survived for equally long periods. The problems encountered by those animals that died after the drastic procedure of direct transfer was apparently due to inadequacies of gut function, either in its absorptive capacity and/or through the production of a constriction in its hind region. It is significant that the swollen abdomen indicative of this situation was never found in acclimated animals. The experiments involving the successful return of animals to fresh-water from full-strength sea-water show that *L. fluviatilis* does not lose its ability to regulate in fresh-water, a feature that presumably reflects the fresh water origin of the group (Morris, 1972). It is of interest that the degree of osmoregulatory ability of down-stream migrant lampreys is greater than that developed by the migrating smolt stage of *Salmo* species investigated by Parry (1960), another group with a fresh water derivation. In *Salmo salar*, the most successful species, the mean duration of survival after direct transfer to 100% sea-water was 84 hours, although 75% could be tolerated for long periods.

Although it is not known from which tributary of the Severn the downstream migrant stages originally came, the close similarity of their mean length (99·9 mm) in 1970 with that of metamorphosing *L. fluviatilis* (98·5 mm) taken from the River Teme in the previous December (Hardisty *et al.*, 1970) suggests that little or no reduction in length occurs during the last few months of metamorphosis. A similar situation has also been found in *P. marinus* by Wigley (1959) and *M. mordax* by Potter (1970) whose studies showed that in comparable phases of the life cycle of these other parasitic lampreys there may even be a slight increase in length even though the animal is not feeding at this time.

The proportional body measurements of these *L. fluviatilis* obtained from elver trawls in 1970 and 1972, which are of virtually identical length to those measured by Hardisty *et al.* (1970) in early December 1969, show that little alteration takes place in most of the body intervals, in contrast to the marked changes during the first months of metamorphosis. There is however, a small but significant reduction in both the body depth and the legnth between the branchial apertures, the former presumably being correlated with the transfer of materials from the body to support the animal during this non-trophic phase. The smaller disc and preorbital region in the 1971 sample appears to reflect a difference between the characteristics of two sub-populations in the catches of this year (Fig. 3).

The sharply defined first peak in the three sets of length and weight-frequency curves (Figs 1 and 2) strongly suggest that the animals in this area of the curve belong to a single year class. As Hardisty & Huggins (1970) found metamorphosing individuals of similar lengths in an ammocoete population from the River Teme in which the average duration of larval life was estimated to be $4\frac{1}{2}$ years, it seems probable that these downstream

migrants are five years old. The small peak to the right of the mid-point of the "trough" in both the length and weight-frequency curves of 1970 and 1972 is also apparent in the Teme sample mentioned above. This part of the curve probably represents animals that have spent an additional year as an ammocoete and would thus be six years old. The situation regarding the similarly located peaks or sub-peaks to the right of the mid-point of the "trough" in 1971 is more problematical. As was shown in Fig. 3, the weight/length relationship of 16 of the larger animals is very different to that of the other members of the population and to the 1970 and 1972 samples. The most plausible explanation seems to be that they represent a group of animals that have come from an area of the Severn whose environment, from a growth point of view, differs from that of other regions. The similarity between the lengths and weights of the 1970 and 1972 samples and those corresponding to the bulk of the 1971 population (Figs 1 and 2) are in this context remarkable as they probably contain individuals from several tributaries. This reflection of an apparently homogeneous growth rate (apart from that of the small anomalous group in 1971) is surprising in view of the variation in growth observed not only between rivers (Zanandrea, 1961) but also between sites on the same river (Hardisty, 1944; Potter, 1970). It seems possible that the relatively low weights of the atypical individuals in 1972 may have been due to their inhabiting an area of reduced food supply. Because metamorphosis is apparently dependent both on age and length (Hardisty & Potter, 1971; Manion, 1971) and on the amount of stored lipid (Lowe, 1972), this particular group may not have deposited sufficient food reserves by the age of $4\frac{1}{2}$ years, when transformation is normally initiated, and they have thus extended their larval life by a year.

Since the adult *L. fluviatilis* caught in trawls from the upper part of the estuary and lower reaches of the river bear the colouration characteristic of early spawning-run stages, it would appear that their migration starts in the spring rather than in the autumn, as is usually the case in the Severn. The significantly shorter lengths of these animals, compared with more typical Severn adults, imply that they feed for a shorter period. Although there has been little detailed investigation on the duration of the parasitic phase of any anadromous lamprey, there is some indication that in *L. fluviatilis* this is approximately 18 months (Zanandrea, 1958; Hardisty & Potter, 1971). It thus seems possible that the spring migrants, of which there are examples recorded from other European rivers (Benecke, 1881; Abakumov, 1961; Gaygalas & Matshevichyus, 1968), have fed in the estuary and/or the sea for 12 months, their migration downstream and upstream occurring in the spring of successive years. Since the large typical adults are already on the spawning grounds in April, it seems very unlikely that breeding of the spring migrants can occur at the same time. A summer spawning season would be consistent both with the reports of small *L. fluviatilis* breeding in June (Hardisty, pers. comm.) and with the large but not fully matured gonads of spring migrants in April.

The information outlined in this paper on the downstream migration of *L. fluviatilis* demonstrates that in this phase of their life cycle, as in other aspects of their biology (Berg, 1935), there are many parallels with the anadromous Salmonidae. For example, in the period prior to this movement, the lamprey becomes increasingly silver in colour and develops relatively large eyes paralleling "smoltification". The migration in *L. fluviatilis*, as in several salmonids (White, 1939; Neave, 1955; Foerster, 1968), occurs in the Spring and is nocturnal with cover or suitable burrowing substrates being sought by day. At the time of migration, the salmon species often show a preference for areas of faster water

flow (Hoar, 1954), a feature also observed by Potter (1970) in the Southern Hemisphere lamprey *M. mordax*. It seems likely that, in both lampreys and anadromous salmonids, one or a combination of such factors as increasing water temperature and day length and marked water flows, are important environmental factors in stimulating and facilitating downstream migration. In view of these parallels in morphology, behaviour and ecology, it would be of considerable interest to know to what extent endocrine changes involved in the seawards migration of teleosts are paralleled by the very distantly related lampreys at comparable phases of their life cycle.

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